Coagulation Timer Unit —

MLA ®

ELECTRA 750

instruction
manual

326364

ELECTRA® 750/750A

WARRANTY

This instrument is warranted to be free, under normal use and
authorized service, from defects in workmanship and material
for a period of one year from the date of installation. The
Photolamp, MLA Catalog #9012, is guaranteed for a period of 30
days. THIS EXPRESS WARRANTY IS IN LIEU OF ALL OTHER EXPRESS AND
IMPLIED WARRANTIES, INCLUDING ANY IMPLIED WARRANTY OF
MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, AND IN LIEU
OF ANY OTHER OBLIGATION ON THE PART OF MLA.

Such express warranty shall not be effective unless this
instrument is serviced solely by service agents authorized by
MLA. MLA shall not be liable for any damage to or defect in the
instrument or for any personal injury or property damage which
may be caused by the instrument subsequent to any servicing by
unauthorized personnel.

This warranty shall be fulfilled by MLA's authorized dealers.
Contact your authorized MLA distributor or service dealer when
help is needed. MLA's Technical Services Department is available
by phone to both customer and dealer when required.

MLA's LIABILITY FOR DAMAGES SHALL NOT EXCEED THE AMOUNT RECEIVED
BY IT FOR THIS INSTRUMENT. IN NO EVENT SHALL MLA BE LIABLE FOR
INCIDENTAL, CONSEQUENTIAL OR SPECIAL DAMAGES, RELATING EITHER TO
PERSONAL INJURY OR TO PROPERTY DAMAGE.

The warranty card, properly filled out with the installation
date, must be returned to MLA.

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SECTION 1

INTRODUCTION

The Electra 750 (E750) is a precision photo-optical plasma coagulation timing instrument that performs routine coagulation tests. In addition, the E750 performs Factor Assays, Quantitative Fibrinogen Assays via a Thrombin Time method, and many other tests in citrate as well as oxalate collected systems. Reagents and controls produced by major manufacturers have been tested and can be used on the E750.

The Photometric method utilizes the change in optical density of plasma to detect a clotting endpoint. When fibrin is formed in the plasma as a part of the coagulation process, the optical density increases. A photocell converts this change in optical density (O.D.) to an electrical signal, which is processed and compared to a reference level. When the processed O.D. signal exceeds a reference level, the clot is detected and the internal timing system displays the clot time on a front panel display.

Precise coagulation testing requires that the variable parameters influencing coagulation be precisely controlled. The MLA photometric system offers some distinct advantages over other methodologies in this area. For example, cross-contamination is eliminated because nothing comes in contact with the specimen. This is also a distinct safety feature because the operator does not have to wipe dirty or contaminated electrode probes.

The Electra's photo-optical system looks at the entire plasma sample and is sensitive only to changes in the overall optical density. Artifacts, due to the presence of foreign bodies or fibrin strands present in the plasma before the coagulation reaction begins, are avoided.

Many useful features have been added to the Electra 750 to provide easier, more accurate and precise measurements. Among these features are an off-scale indicator that lights if the specimen density exceeds the instrument optical range; a heating block that maintains samples, pipettes, tips and reagents at $37°C \pm 0.2°C$; reagent reservoir with magnetic stirring, and special pipettes that automatically start the clot-timing circuitry when the reagent is added to the patient sample.

The following sections of this manual describe in detail the installation, operations and maintenance of the Electra 750. We suggest that you take the time to read this manual in order to get the best performance possible from this instrument.

SECTION 2

TABLE OF SPECIFICATIONS
ELECTRA 750

PHYSICAL
SIZE: 10 7/8" (27.6cm) wide x 17 5/8" (44.9cm) long x 6 3/4"
          (17.2cm) high
Weight   27.8 lbs. (12.6kg)

POWER REQUIREMENTS
  Max power dissipation (Heater ON): 115 watts
  Input voltage ranges (internally selectable)
        90-110 VAC
        110-125 VAC
        210-230 VAC
        230-250 VAC

  Line frequency: 50 or 60 Hz (rear panel switch selectable)
  Fusing: for 90-125 VAC: 2A
            for 210-250 VAC:1A

TESTING
  Test Modes   PT
                 APTT
                 THROMBIN
                 SPEC 1
                 SPEC 2

  Timer Resolution: 0.1 seconds
  Maximum Time Readout: 999.9 seconds
  Minimum Clot Time: This varies with the Mode
                 PT   8.4 seconds
                 APTT   16.5   "
                 THROMBIN 2.5   "
                 SPEC 1   8.4   "
                 SPEC 2   16.5   "

  Test Method: Photo-optical clot detection.
                 Second derivative processing.

  Test Performed: PT,APTT, Certain Factor Assays, Quantitative
                     Fibrinogen Assays, and other Coagulation
                     Tests. (See Section 8).

  Test Performance: Performance varies greatly depending upon
                     the test, method, reagents, etc. In clinical
                     trials performed by MLA, coefficients of
                     variation of 0.5% for PT, and 0.6% for APTT
                     were obtained. See Section 8 for notes on
                     specific tests and procedures. Further data
                     based on tests performed at MLA and in
                     hospital labs, are available on request.
                     Reagents and controls produced by major
                     manufacturers have been tested satisfactorily
                     on the E750.

INCUBATION TIMER
    Resolution: 0.1 second
    Operation: Push Button start/stop. Reset by test cuvette
                 in the test station.

HEATER BLOCK/WORK STATION
    Temperature Regulation $37.2°C \pm 0.2°C$

    Capacity: 10 Test Incubation Stations
                      2 Reagent Incubation Wells (One Reagent Well
                        has Magnetic Stirrer)
                     50 Pipette Tips
                      2 Instrument Pipettes

    AT TEMP Indicator: Lights when block temperature is within
                         $\pm 0.3°C$ of factory set temperature
                         Range $25° - 45°C$
                         Accuracy $\pm 0.5°$ from $30°$ to $40°C$

The Heater Block is operational as long as the instrument is
connected to a power source.
The heater system is protected by a thermal fuse.

INSTRUMENT PIPETTES
    0.1 ml and 0.2 ml $\pm 5\%$

SAFETY
    Maximum leakage current to ground - 325 microamperes @ 120
    volts, $25°C$.
    High voltage breakdown - withstands 900 VAC from line to
    ground for 1 minute.

RADIO FREQUENCY INTERFERENCE (RFI)
    An RFI filter is included to minimze susceptibility and
    emission of RFI.

SECTION 3

UNPACKING AND REPACKING

UNPACKING

·The Electra 750 is packaged in a single carton with a Starter
Kit. Unpack the instrument carefully noting position of
materials. See Figure 3-1. Remove entire unit plus packaging
by pulling straight up on the ends of the packing material.

NOTE

SAVE ALL PACKING MATERIAL FOR USE IF REPACKING
BECOMES NECESSARY. SHIPPING IN UNAUTHORIZED
CONTAINER IS CAUSE FOR VOIDING WARRANTY.

Check packing list to determine that all items are included.
Disposable supplies should be ordered immediately to avoid being
without supplies when those in the starter kit are exhausted.

PACKING LIST FOR ELECTRA 750 - CATALOG NUMBER 1041

QTY.	DESCRIPTION	REORDER PART NO.
1	Coagulation Timer	A15E200
1	Instrument Manual	326364
1	Graph Pad	9041
1	Warranty Card	----
1*	Pipette Tips in Tray	9620
1	Starter Kit, consisting of:	A15C426
80*	Cuvettes	9005
100*	Pipette Tips	
1	0.1 ml Precision "D" Pipette	1055
1	0.1 ml Electra Pipette	1016
1	0.2 ml Electra Pipette	1017
1	Line cord	14992
1	Thermometer	A15A424
1	Magnetic Stirrer	9006
2	Beaker	9004
2	Beaker Cover	9008

* When these items are reordered, quantities larger than included
in this kit are provided.

REPACKING

When the Electra 750 must be shipped, the same packing material in
which the unit was received must be used. Prior to repacking,
remove the workstation thermometer by sliding a piece of paper under
the thermometer as shown in Figure 3-2. Pull straight up on the
paper and lift the thermometer clear of its well.
If it is necessary to return the instrument to the factory, be
sure to include the thermometer, pipettes, and other loose material
(i.e., line cord, reservoirs, etc.)

Starter Kit

Foam Packing

ELECTRA 750,750A

Foam Packing

Instruction Manual

Shipper
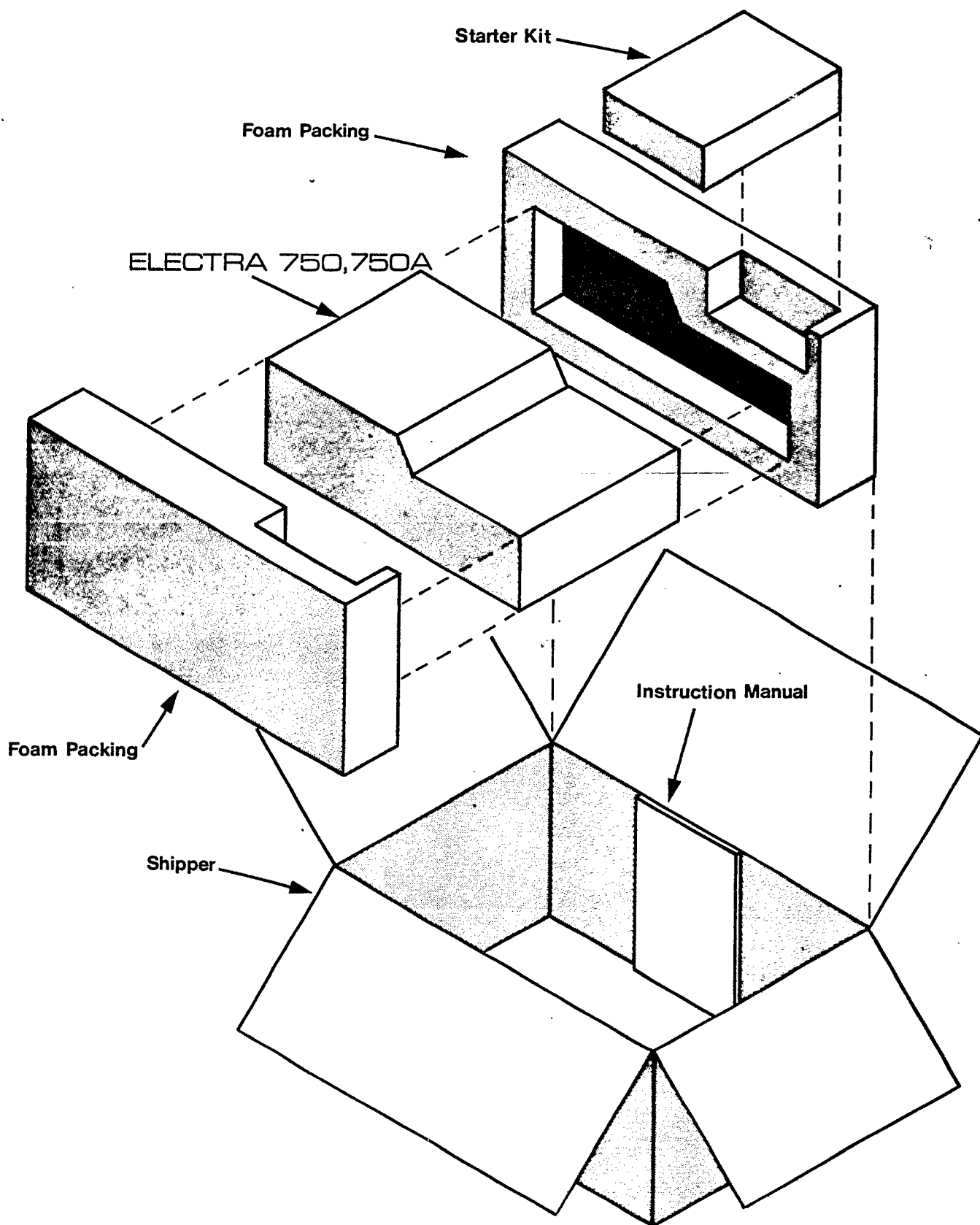
3-1   Packaging for the Electra 750

DIRECTION OF PULL

PULL

PAPER

THERMOMETER

CROSS SECTIONAL VIEW
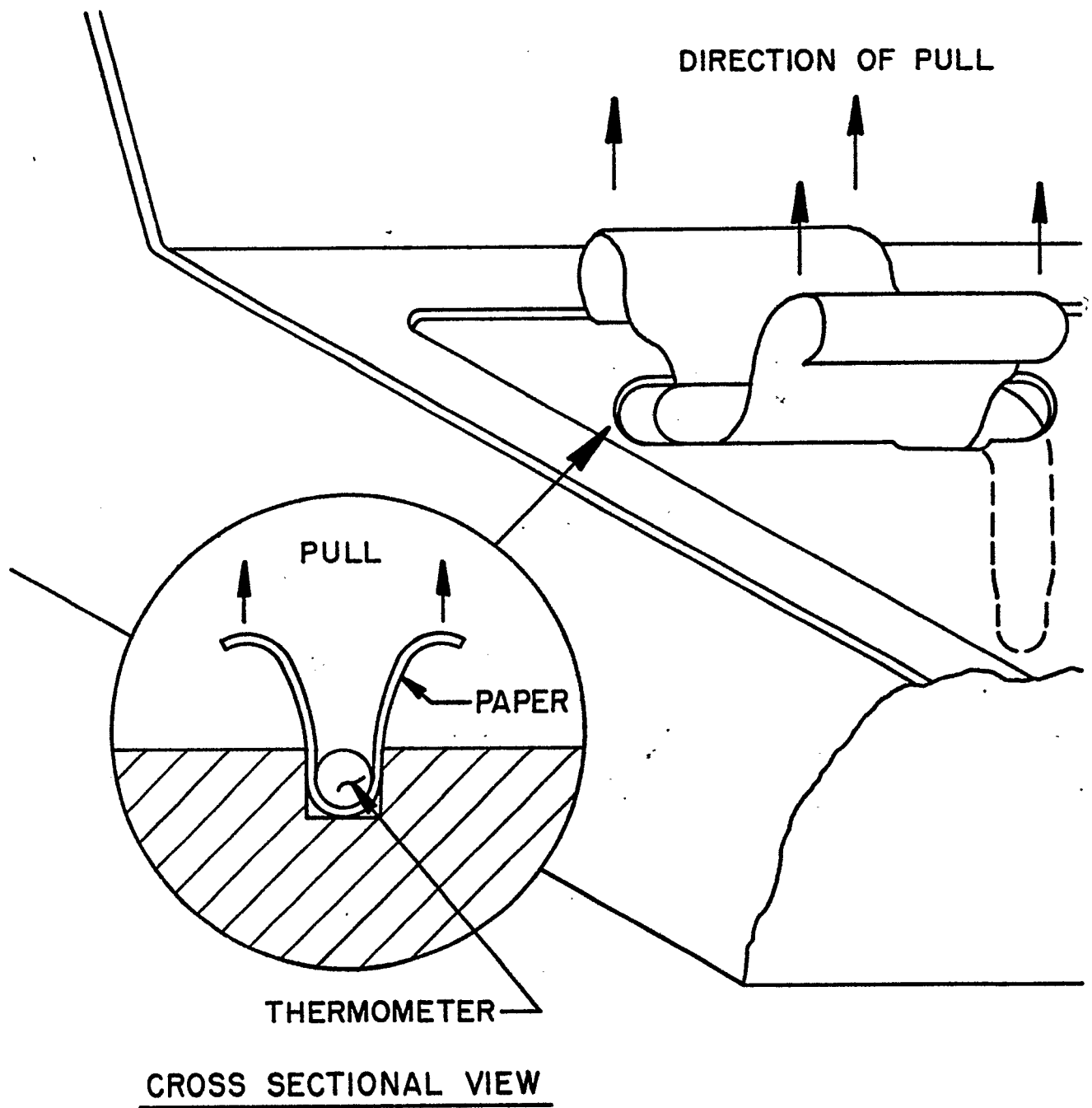
Figure 3-2   Removal of Workstation Thermometer

SECTION 4

INSTALLATION

INTRODUCTION

This installation section provides information covering initial
testing and confidence check procedure to insure that the
instrument is working properly. If the instrument does not
function or an anticipated result is not obtained, refer to
Section 10, Troubleshooting.

Table 5-1 should be consulted prior to installation. (See Section 5)

INSTRUMENT INITIAL SET-UP

The following one-time procedure must be performed after unpacking
and prior to instrument use.

NOTE

> BEFORE CONNECTING THE INSTRUMENT TO A POWER SOURCE,
> CHECK THAT THE POWER SETTING ON THE REAR PANEL
> AGREES WITH THE POWER LINE VOLTAGE AND FREQUENCY
> AT YOUR FACILITY.

Domestic U.S. models are normally shipped to operate on 110-125
volts, 60 Hz. Overseas models are shipped to operate on 210-230
volts, 50 Hz. If your line voltage is within the range indicated
on the back panel, (See Figure 5-1) skip to step h. If it is not,
perform steps a through g. If your line frequency differs from
the frequency indicated on the rear panel, use a small screwdriver
to set the Hz switch on the rear panel to the proper frequency
(either 50 or 60 Hz).

BE SURE THE LINE CORD IS DISCONNECTED BEFORE PERFORMING FOLLOWING STEPS.

a)   Remove instrument cover by unscrewing 8 screws. Locate the
     barrier strip on the rear panel, right side. (See Figure 4-1.)

b)   Remove two hex nuts and fiber paper.

c)   Loosen two screws holding shorting bar and remove shorting bar.

d)   Place shorting bar in position so that the correct voltage
     range marking is visible through the cut-out in the rear panel.

e)   Loosen associated barrier strip terminal screws and place
     shorting bar on required terminals and tighten screws.

f) Replace fiber paper and hex nuts.

g) Replace cover, check fuse value (should be 2A for 90-125 or 1A for 210-250).

h) Install the power cord and connect it to a power source. The heater for the incubator block is now operational and will begin to raise the block temperature to 37°C. The block will maintain its temperature at 37°C as long as the power cord is connected to the power source. Remove the right angle thermometer from the protective packaging and refer to Figure 4-2. Install the thermometer in the thermometer well on the top left side of the heating block, checking that it snaps into place.

i) Set up the work station by putting reservoirs, tips, cuvettes and pipettes in place as shown in Figure 4-3. The magnetic stir bar will rotate in the reagent reservoir after the power switch has been turned ON.

j) Operate LAMP LEVEL switch on rear panel to B.

The E750 is provided with two special pipettes, a o.1 ml and a 0.2 ml, two stainless steel reservoirs and an area for storing 50 disposable pipette tips. These items are all contained in a heated area (37°C±0.2°C) of the incubator block. The reservoirs are used to contain reagents and calcium. The reagent reservoir has a magnetic stir bar that rotates to keep particulate reagents in suspension. Both reservoirs are fitted with plastic covers to help retard evaporation, maintain temperature and keep out foreign matter. Provision is made for a tray to hold 50 disposable pipette tips in the workstation. Replacement pipette tips in prepackaged trays are also available (Order No. 9620). Tips maintained in these trays in the heated area provide warm tips for aspirating warm reagents. This action provides greater accuracy by not cooling reagent prior to mixing with heated plasma under test. The pipettes are also heated and are designed to fit the test station, dispense the reagent, and initiate clot timing. These pipettes also provide a light shield for the specimen under test.

INITIAL TESTING (OPERATOR CONFIDENCE TEST)

This procedure is performed when the instrument is initially installed or when the instrument has been moved to a new location. This Operator Conficence Test simply provides a means by which the operator can determine that the instrument is performing the required functions in a satisfactory manner. When the desired result is not achieved, refer to Troubleshooting, Section 10. To conduct this test, refer to Figure 4-3 and perform the following:

a) Connect E750 to power source. See paragraph 4.2.

b) Turn on the unit by operating power switch to ON. Digital display should read "0".

c) Remove test cuvette (if any) from test station. Press INCUBATION TIMER switch. Check that digital display counts. Press INCUBATION TIMER switch again and check that digital display resets to "O".

d) Restart Incubation timer by pressing INCUBATION TIMER button and, while it is timing, place test cuvette in test well. Check that timer resets to zero and does not operate unless cuvette is removed from test station.

e) Obtain stopwatch. Simultaneously press INCUBATION TIMER and start stopwatch. Check times on both 750 and stopwatch after 60 seconds to see that timers agree. If stopwatch and timer disagree, line frequency switch may be set incorrectly. Reset switch.

f) Place empty test cuvette into test station and place either instrument pipette over test cuvette.

g) Set LAMP LEVEL switch, on rear panel, to A (up position). Set Mode switch to PT.

h) Press pipette plunger and observe digital display. Check that timer counts to 7.0 seconds, then, resets to zero. Check that OFF SCALE indicator then lights.

i) Remove cuvette from test station and check that OFF SCALE indicator goes off.

j) Repeat Step (h) for each of five mode settings. Time at which display resets varies with mode setting. This time is guard interval. The following table provides guard interval for each Mode.

TABLE 4-1 GUARD INTERVALS

MODE	GUARD INTERVAL
PT	7 seconds
APTT	16 seconds
THROMBIN	2 seconds
SPEC.1	7 seconds
SPEC.2	16 seconds

COVERED BARRIER STRIP

FIBRE PAPER

HEX NUTS

SHORTING BAR
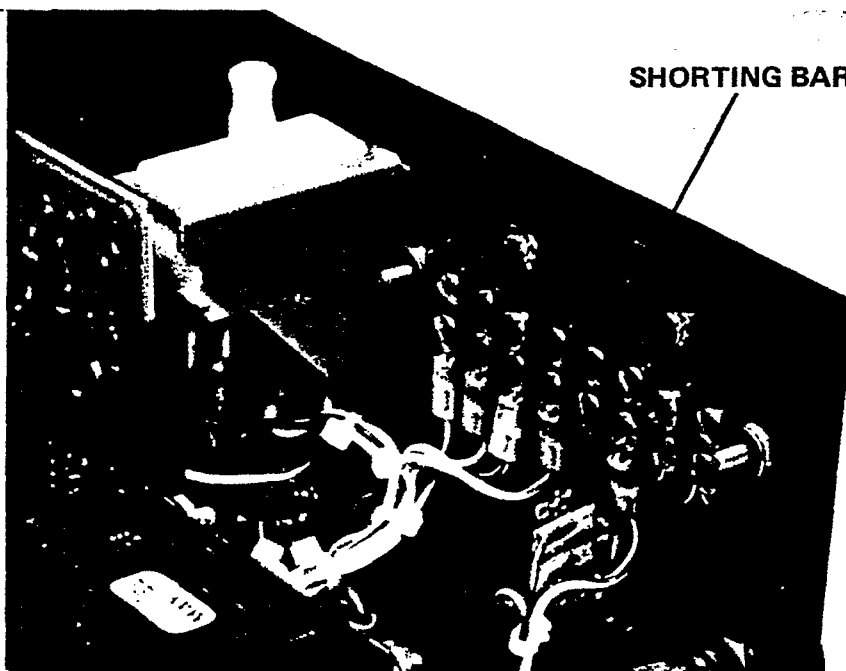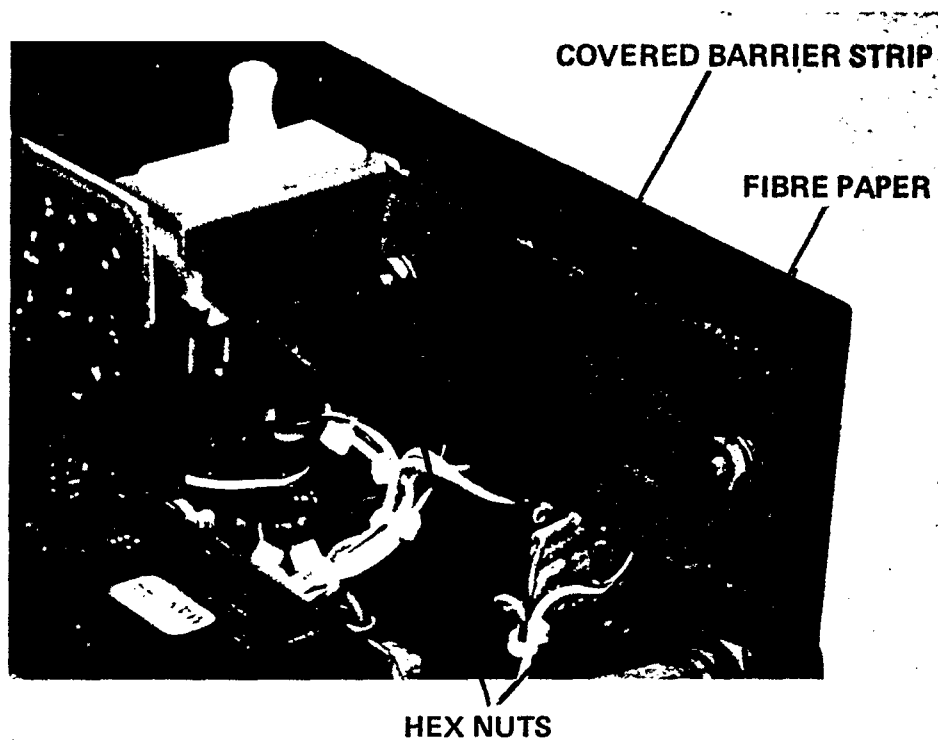
Figure 4-1   Primary Voltage Adjustment Location

DO NOT PUSH HERE

PUSH HERE

9

10

7

8
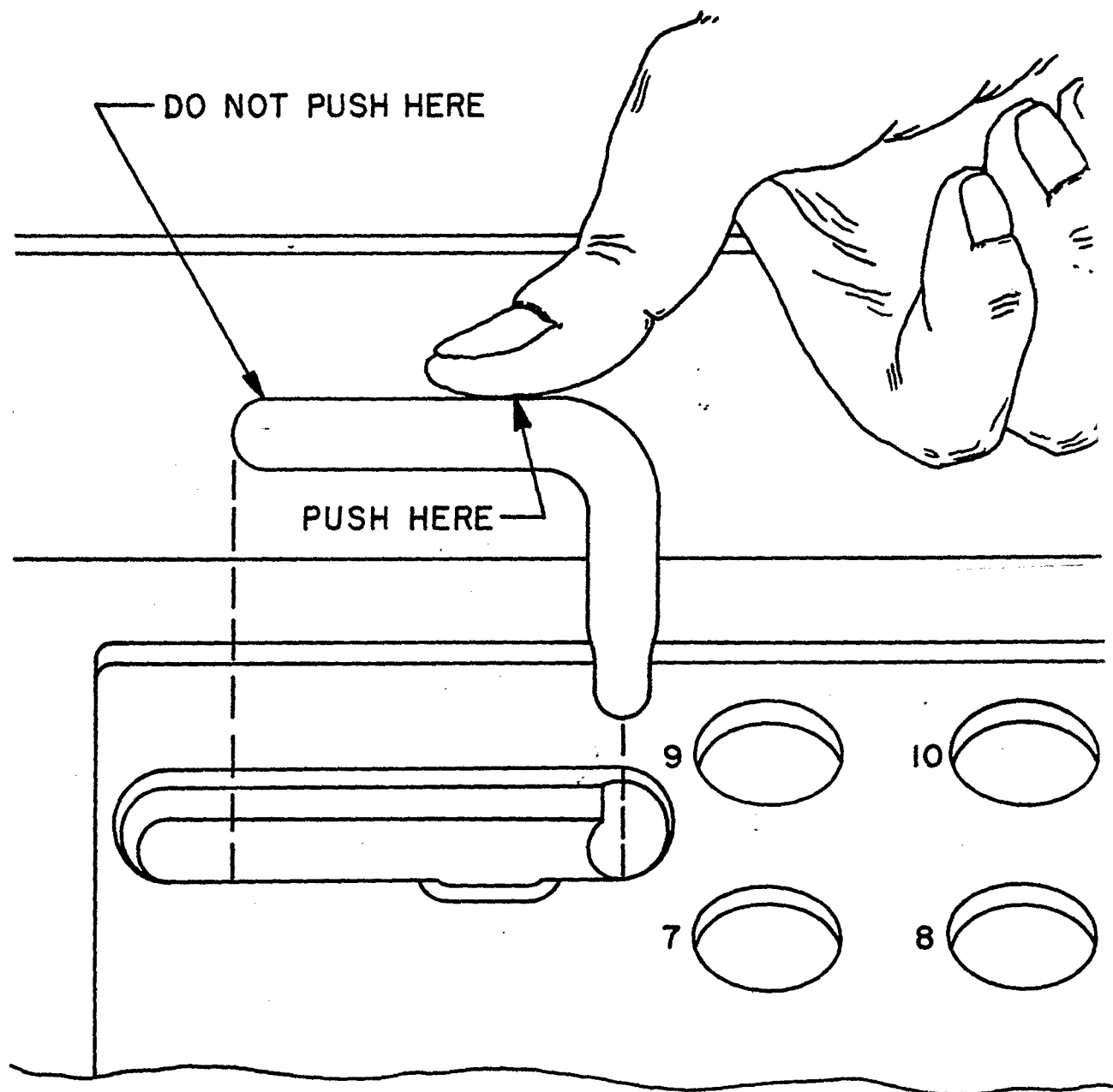
Figure 4-2   Installing Workstation Thermometer

Figure 4-3   Electra   Workstation

k) Set LAMP LEVEL switch to C (down position). Set Mode Switch to APTT. Place empty cuvette in test station and cover with pipette.

l) Push pipette plunger to start timer. While timer is running, operate LAMP LEVEL switch from C to B and back to C, prior to and after the expiration of the guard interval. When the E750 is operating properly, this simulated clot signal does not stop timing prior to the guard interval. However, operation of the switch after completion of the guard interval will stop the timing. The displayed time should be equal to or longer than the guard time indicated in Table 4-1 for that mode.

m) Check that temperature on workstation thermometer reads $37.2°C\pm0.5°C$.

<u>NOTE</u>

Workstation temperature is factory set at 37.2°C using an extremely accurate low mass temperature sensor. Small bulb thermometers and other such devices are not very accurate. Do not request service if your temperature is measured on an external device and disagrees with workstation thermometer by less than 1°C.

n) Place magnetic stir bar in REAGENT reservoir and check that it rotates.

The preceding activity constitutes the preliminary installation procedure. Refer to Section 5, then to Section 8 to perform a Prothrombin Time Test on control plasmas to complete the installation.

SECTION 5

OPERATION

INTRODUCTION

This section covers the general operation of the instrument.
Refer to Section 8, Coagulation Testing, for specific tests and
procedures for each test.

CONTROLS, INDICATORS

Table 5-1 describes the function of E750 controls and Figure 5-1
shows location of these controls and indicators. (Table 5-1 and
Figure 5-1 are at the end of the section).

INSTRUMENT TURN-ON

The instrument turn-on procedure is an abbreviated Operator
Confidence Test, and can be conducted by performing the following
steps:

a) Operate ON-OFF switch to ON, lamp level switch to B, mode
    select switch to PT, and check that the AT TEMP indicator
    is lit.

b) Place an empty cuvette and instrument pipette in place at
    test station, and press pipette plunger. Check that the
    OFF SCALE indicator remains extinguished for at least 10
    seconds as indicated on TIME display.

c) Remove and reinsert the empty cuvette, replace instrument
    pipette, operate lamp level switch to A, and press pipette
    plunger. Check that: OFF SCALE indicator lights after
    approximately 7 seconds, and TIME indicator automatically
    resets to 0 after the count. If OFF SCALE indicator does
    not light, see Section 10.

d) Check that workstation thermometer indicates 37°C±0.5°C.

PREPARATION FOR TEST

Prepare the instrument for testing by performing the following
steps:

a) Place sufficient amount of reagent in reservoir. Keep
    covered to maintain temperature. Allow 20 minutes per
    10 ml for heating to 37°C.

NOTE

Only REAGENT reservoir has magnetic stir
bar for particulate reagents that require
continual mixing.

b) Place tray of MLA disposable tips in position shown in Figure 5-1. These tips will be heated and should be used in aspirating reagent and calcium for tests.

c) Select mode of operation.

d) Incubate sample by placing specimen under test in incubation station at staggered intervals to provide incubation for length of time specified by type of test. Time for the normal sample to clot plus time required to remove clotted specimen and replace with another specimen at staggered intervals. This action prevents over incubation of specimens to be tested. Incubation timer may be used to time incubation period.

e) After incubation, place sample in test station, pushing cuvette to bottom of test well. This action will reset timer to zero if it was being used to time incubation period.

f) Using appropriate E750 pipette (red or blue cap), pick up prewarmed disposable pipette tip, aspirate reagent and place over cuvette in the test station. Align pipette to have flat side containing marking towards you.

g) Push plunger firmly and hold down for one second, then release plunger. LEAVE PIPETTE IN PLACE UNTIL TEST IS COMPLETED. DO NOT DISTURB PIPETTE DURING TEST.

h) Watch completion as indicated by timer displaying a fixed number. If during test, OFF SCALE indicator lights, remove sample from test station and observe its density. If this sample appears more opaque or darker than previous successfully run samples, operate LAMP LEVEL switch on rear panel to B or A to increase brightness of lamp. If sample appears more transparent or lighter than previous successfully run samples, operate LAMP LEVEL switch on rear panel to B or C to reduce brightness of lamp. If OFF SCALE indicator continues to light, photolamp replacement may be required, refer to Section 10, Maintenance.

NOTE

When the OFF SCALE indicator lights, the specimen under test is outside the range of the scale on which the instrument is operating. The density of this specimen is either too dark (dense) or too light (transparent). When this occurs, the timer will reset to zero. For an off-scale condition, the OFF SCALE indicator will light at some interval of time after the start of the test, depending on the mode of operation. See chart below:

MODE	TIME (SECONDS)
PT	7
APTT	16
THROMBIN	2
SPEC.1	7
SPEC.2	16

i) Upon completion of test, record clot time and discard cuvette and pipette tip.

CAUTION

Use a clean disposable tip on a blue or red topped instrument pipette for each test to prevent sample carry-over to the reagent reservoir.

NOTE

Inserting next specimen will reset timer to zero automatically.

CONSIDERATIONS PRIOR TO TESTING

Three areas of activity influence results: (1) Materials used, (2) Operator techniques and (3) Instrument status.

MATERIAL

PIPETTES
Defective pipettes or tips that cause improper volume dispensing, will alter clot times.

IMPROPERLY WASHED TEST TUBES
Rewashed test tubes may contain detergent remnants which will have a destructive effect on plasma samples.

AGING REAGENTS
Frequently, reagents are improperly stored, or exceed their manufacturer's shelf life specifications. Aging or defective reagents generally prolong clot times and can cause poor duplicate times.

CONTROL TIME DEVIATIONS FROM LOT TO LOT
The Electra will detect differences between different lots of controls and controls that have been aged. Controls are also sensitive to improper storage and aging, just as reagents. Poor duplicate times may result from controls that have been handled improperly.

pH LEVELS OF REAGENTS

a) <u>Water</u>: Distilled and Deionized water or equivalent, pH=6.0-7.0. Reconstituted reagents should have a pH of 7.2-7.4.

b) <u>Saline</u>: 0.9 to 0.95% Sodium Chloride solution buffered to pH 7.3-7.4 (i.e. Owren's Veronal Buffered, Imidazole Buffered).

c) <u>Blood drawing tubes</u>: Borosilicate glass, sterile interior, 3.8% or 3.2% Sodium Citrate.

CENTRIFUGING OF PATIENT SPECIMENS

Specimens must be adequately centrifuged, otherwise both the precision and accuracy of replicate times may be altered. Specimens should be spun for 15 minutes at 1000g.

OPTICAL CONSIDERATIONS OF REAGENTS

Some types of reagents, such as Kaolin, are of such a dense nature that they exceed the ability of the Electra's optical system to <u>see through</u> the specimens. Consequently, such material is not suitable for use on the Electra. If a reagent specimen mixture is too dense for the instrument, the OFF-SCALE indication will light.

OPERATIONAL TECHNIQUE

Technique can have a marked effect upon results, especially in those tests where multiple reagents are used, such as: APTT. It is important, therefore, to observe the following:

1. Pipette precise and correct quantities of all liquids.
2. Pipette specimen and reagent directly into the bottom of the test cuvette. (Sample should not be discharged along the sides of the cuvette.)
3. Provide uniform and exact incubation times, according to MLA Operator's Manual. See section 8, for the specific test.
4. Reconstitute controls in accordance with manufacturer's instructions and allow to stand for at least 15 minutes.
5. Make certain that reagents specified for use at 37°C be at that temperature. (Cold reagents placed into Electra reservoir will require at least 20-30 minutes to reach operating temperatures.)
6. Use controls only on tests for which they are designed. (For example, some controls are suitable only for PT tests.)
7. Do not place the Electra adjacent to machines that can generate extraordinary line surges, electrical interference, or high vibration levels.

INSTRUMENT TEMPERATURE LEVELS

The incubator block/workstation is designed to maintain the test tube incubation wells, the reagent storage reservoirs and the test station at 37°C. This means that the test specimen will be maintained at 37°C during the test cycle providing that proper testing protocol is followed.

For example, although the system may be operating at proper temperature, the following circumstances can cause improper incubation, or cause the specimen not to be at proper temperature.

 a) Cold reagents were put into the reservoir.

 b) Duration of incubation interval has been altered by the operator from those prescribed in the Operator's Manual.

Both of these conditions will change the specimen temperature and may cause erratic results or poor duplication.

VOLUMES

The ratio of plasma to reagents in clotting specimens is important. If either the pipetting device used in preparing the specimens, or the device used to dispense the reagent deviates significantly from the prescribed volume, clot times can be either prolonged or foreshortened significantly.

The pipettes supplied with the Electra together with proper MLA tip, are calibrated to deliver 0.1 ml or 0.2 ml. A defective pipette or defective tip can alter the volume or the quality of the injection stream which will affect results. It is very important that quality MLA pipette tips be used with the Electra 750 pipettes.

TIME (SECONDS)

INCUBATION

12.8

TIMER

AT TEMP

THROMBIN

APTT — SPEC. 1

PT — SPEC. 2

ON

OFF

[MLA] ELECTRA 750
— COAGULATION TIMER —

0.1 ML PIPETTE

PIPETTE WELL

[MLA]

RESERVOIR
WITH MAGNETIC STIRRER

RESERVOIR

PIPETTE TIPS (50)

CUVETTES (10)

0.2 ML PIPETTE
IN
TEST STATION

1 2 3 4 5 6
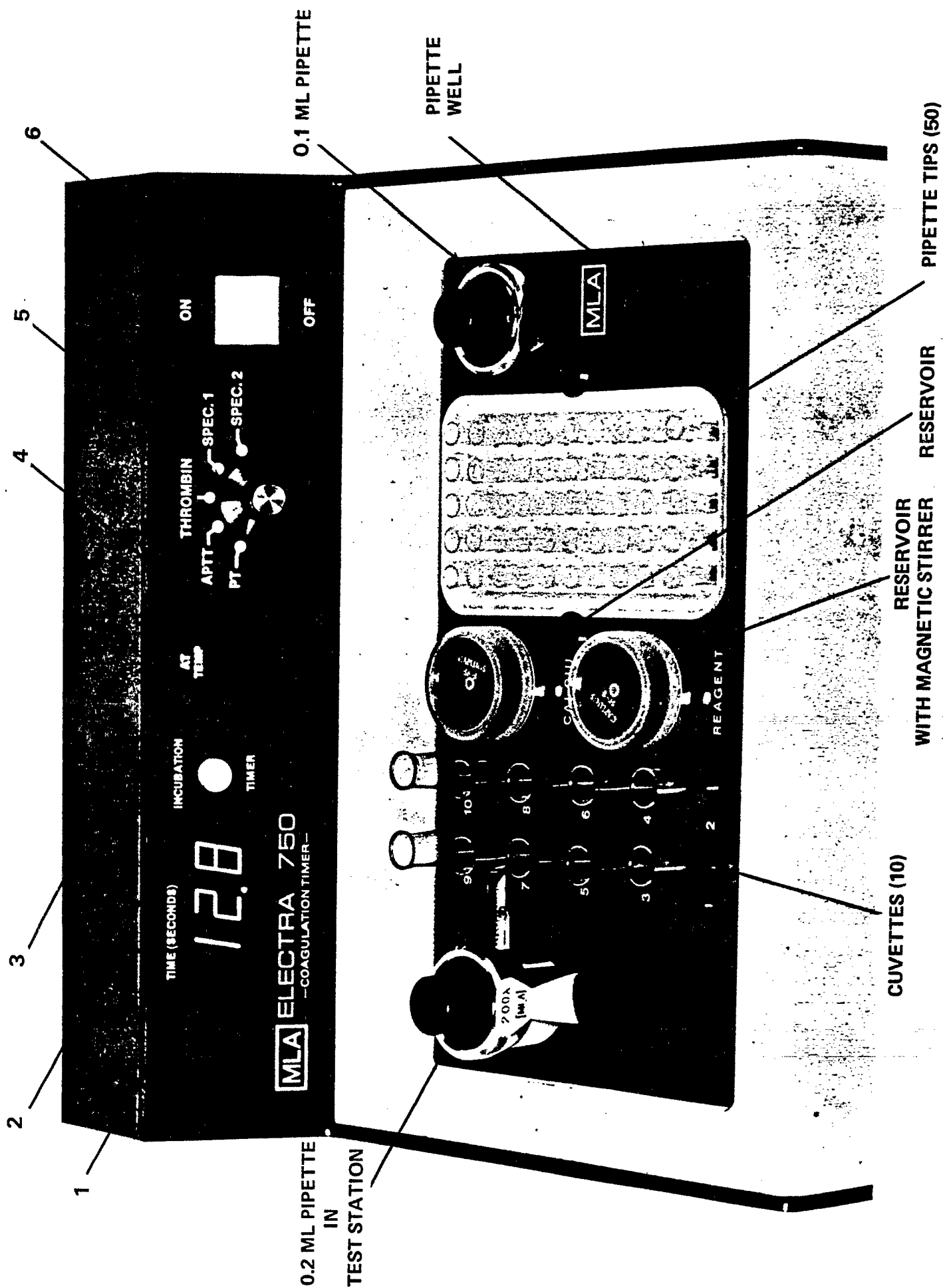
Figure 5-1   Controls, Indicators, and Appliances (Sheet 1 of 2)
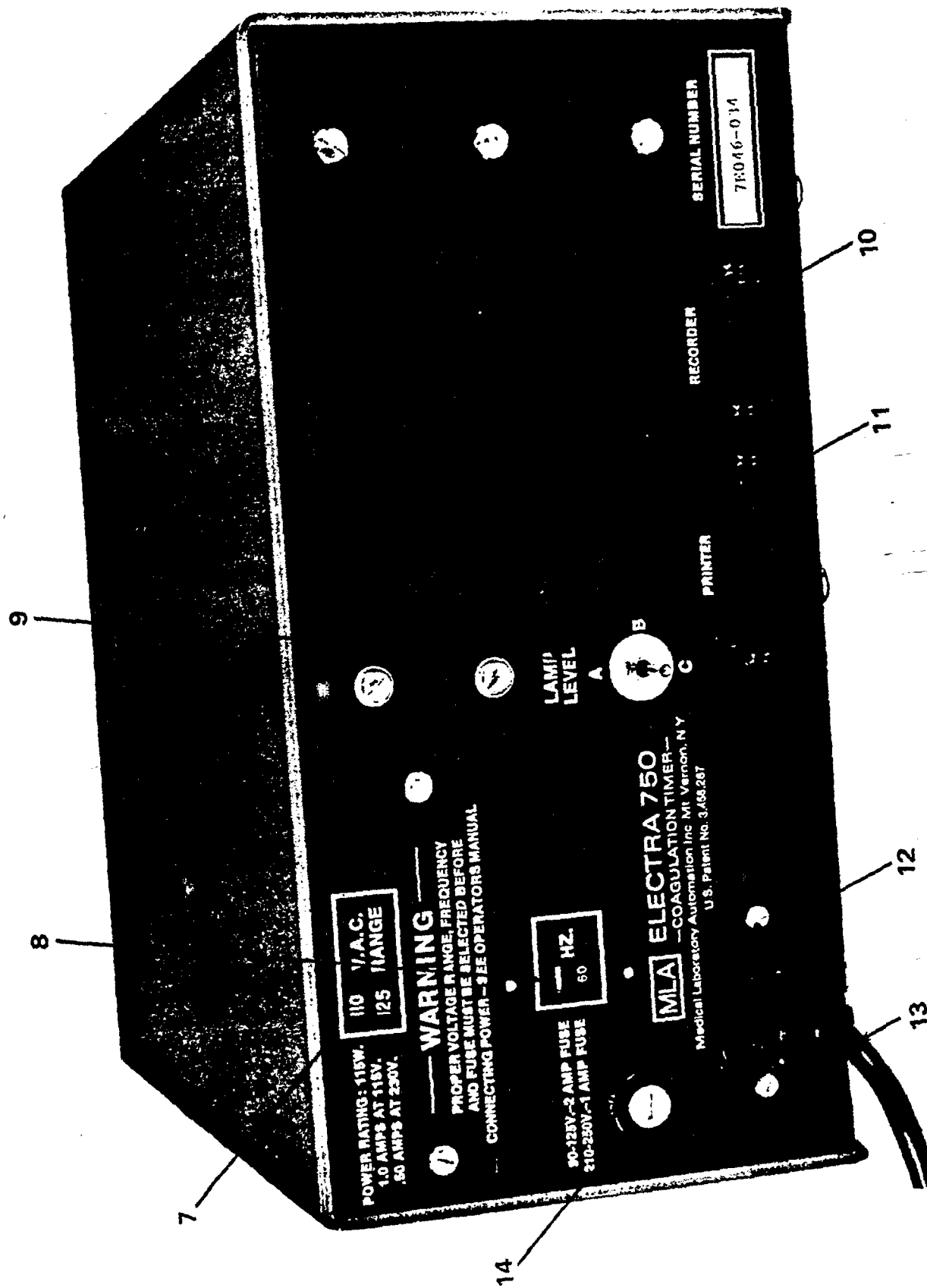
Figure 5-1   Controls, Indicators, and Appliances

TABLE 5-1. OPERATING CONTROLS, INDICATORS, AND APPLIANCES

		FRONT PANEL	
KEY	PANEL MARKING	DESCRIPTION	FUNCTION
1	TIME (SECONDS)	Display	Display clot time or time of incubation.
2	INCUBATION TIMER	Pushbutton Switch	Starts and stops timing when there is no tube in the test station. When timing is stopped, display automatically resets to zero ("0").
3	AT TEMP	Indicator	Lights when incubation heating block temperature is at factory set level $(37.2^{\circ} C \pm 0.2^{\circ})$.
4	OFF SCALE	Indicator	Lights when plasma density is such that clots will not be detected due to out of range condition. Either too dense or too transparent.
5	MODE SWITCH	Rotary Switch	Determines mode of operation.
	PT		Prothrombin Time Test
	APTT		Activated Partial Thromboplastin Time Test
	THROMBIN		Thrombin and Fibrinogen Tests
	SPEC.1		Provides special test parameters to be used as designated by MLA representatives or distributor's salesman.
	SPEC.2		Same as above for SPEC.1.
6	ON-OFF	Rocker Switch	Provides initial primary power to the instrument.

		REAR PANEL	
KEY	PANEL MARKING	DESCRIPTION	FUNCTION
7	V.A.C. RANGE	Window for jumper position indicator	Indicates the range of primary voltage: 90-100, 110-125, 210-230, 230-250.
8	HZ	Slide Switch	Indicates switch position of primary power frequency, which is either 50 or 60 HZ.
9	LAMP LEVEL* A B C	Toggle	Selects intensity of Photolamp to one of three levels: A - Brighter B - Medium C - Dimmer
10	RECORDER	Connector (15-pin)	Provides connection for strip chart recorder to record density and acceleration signals.
11	PRINTER	Connector (25-pin)	Provides a connection for producing hard copy data out on MLA optional printer.
12	AC CONNECTOR	Connector	Connects AC line cord to unit from power source.
13	AC LINE CORD	Cable	Connects AC power to unit.
14	FUSE 1A 2A	Fuseholder	Provides current protection: For 210 to 250 volts For 90 to 125 volts

PIPETTE TIPS

Pipette Tips play an important role in the proper operation of the 750. The mixing action between the patient plasma and reagent is performed by the stream of reagent from the pipette tip. A good stream is shown in Figure 5-2A. When this stream is not directed into the bottom of the tube (see Figure 5-2B) due to poor quality tip, improper mixing occurs and the clot time is adversely affected. MLA 9620 Tips are designed and quality controlled to provide this proper mixing action. Use only MLA Tips for best results.
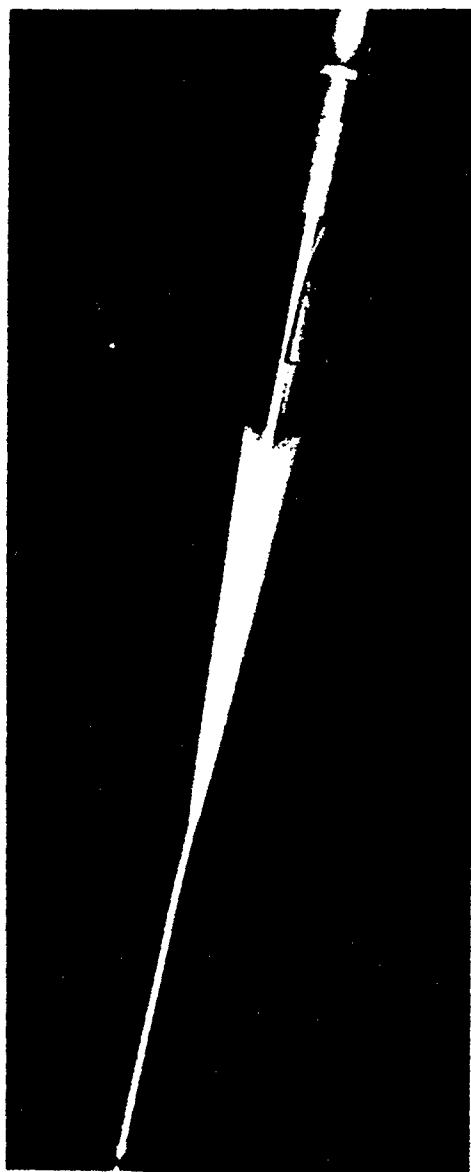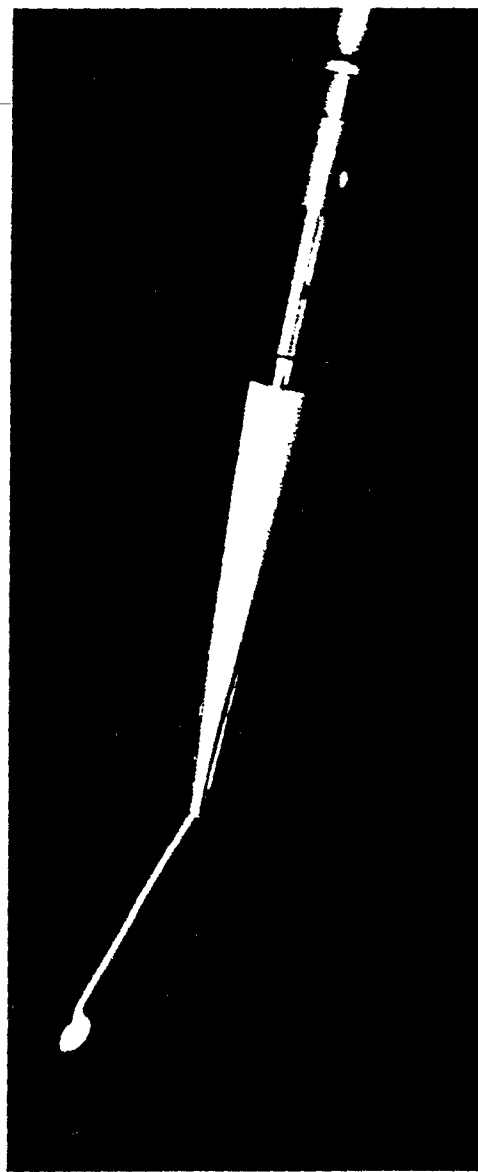
A — MLA TIP   B — BRAND "X" TIP

Figure 5-2. Pipette Tip Quality Comparision

WORK STATION

ITEM	DESCRIPTION	FUNCTION
Thermometer	Mercury glass thermometer.	Indicates temperature of incubation block.
Test Station	Well for inserting test specimen.	Test well for aligning specimens in light path to determine clot time.
Incubation Stations	10 holes in heating block.	10 stations for heating specimens prior to testing.
Cuvettes	Plastic 10x75 tubes with flat bottom. Available as MLA Part No. 9005.	Contain plasma under test for incubation and testing.
Reservoirs	Stainless steel container with plastic top. Only REAGENT container is outfitted with magnetic stir bar.	Holds reagent and calcium for tests.
Disposable Tips & Tray	MLA disposable tips in 50 - count tray (MLA Part No. 9620).	Holds disposable tips in a heated area to provide warm tips. Tips used to dispense reagent.
E750 Pipettes	Blue Cap - 0.1 ml short pipette and Red Cap - 0.2 ml short pipette.	Provides means of aspirating and dispensing reagent, starting clot timing, and providing a light shield for specimen under test. Must be used to initiate test and remain in place during test.
Pipette Well	Two holes in heated workstation to hold pipettes.	To hold and maintain pipettes at approximately 37°C.

*LAMP LEVEL Switch: This switch is used for providing the necessary light level for various specimen optical densities. When an insufficient or an overwhelming amount of light is present, the OFF SCALE indicator lights and an adjustment of the light level is required. This adjustment is made with the LAMP LEVEL switch.

FUNCTIONAL THEORY OF OPERATION

INTRODUCTION

The Electra 750 utilizes the change in optical density of coagulating plasma to determine the coagulation end point. Seven major functions are performed in the 750 to provide an accurate measurement of the clotting time.

THEORY OF OPERATION (Figure 6-1)

The operation of the E750 involves a step-by-step cycle that takes place for each test. This cycle includes reagent injection, passage of time for a guard interval, looking for the clot, clot verification and display of the clot time. Figure 6-1 shows the order and relationship of these activities. The cycle is started by manual operation of the reagent injection button on the E750 Pipette. This action starts the clot timing (See Figure 6-2). After the guard interval (See Table 6-1) passes, the instrument observes the sample for incipient clotting activity. When this occurs, the clot signal is tested and verified; then, the timing is stopped and the elapsed time is displayed.

DESCRIPTION OF OPERATION (See Figure 6-3)

The 750 system is comprised of a photolamp and photocell, an amplifier, a clot timer, a display, an AC control circuit, a heater system, and a power supply system. The 750 system operates utilizing these functions, as follows: A test sample, heated to 37°C is placed in a test station that is located in the light path between a photolamp and photocell. A special pipette is used to add reagent to the specimen to precipitate clotting and initiate clot timing. This action causes the intensity of light impinging on the photocell to be reduced. A signal is developed by the photocell that is related to the magnitude and rate of change of the specimen optical density. The onset of clotting is detected when the rate of change of this signal exceeds a predetermined level. The elapsed time or clotting time is displayed on the front panel. State-of-the-art logic circuits provide control for the start, incubation timing, clot timing, and display functions.

Primary AC power is applied through an AC control circuit to an independently operated heater system for incubation, and to a power supply circuit that converts the AC to low voltage DC. The independent operation of the heating system permits the heater to operate with the power switch OFF, thereby requiring only a five minute warmup for the electric circuits to stabilize.

```
                    ┌─────────────────────┐
                    │       MANUAL        │
                    │  REAGENT INJECTION  │
                    │  CLOT TIMING STARTS │
                    └─────────────────────┘
                              │
                              ▼
                    ┌─────────────────────┐
                    │   GUARD INTERVAL    │
                    │        TIME         │◄──────────┐
                    │  (WAIT)SEE TABLE 6-1│           │
                    └─────────────────────┘           │
                              │                        │
                              ▼                        │
                           ╱     ╲                     │
                          ╱   IS   ╲        NO         │
                         ╱  GUARD    ╲───────────────┘
                         ╲ INTERVAL  ╱
                          ╲  OVER   ╱
                           ╲   ?   ╱
                            ╲     ╱
                              │ YES
                              ▼
              ┌──────►┌─────────────────────┐◄───────┐
              │       │   LOOK FOR CLOT     │         │
              │       └─────────────────────┘         │
              │                 │                      │
              │                 ▼                      │
              │              ╱     ╲                   │
              │             ╱  HAS  ╲       NO         │
              │            ╱ CLOT BEEN╲──────────────┘
              │            ╲ DETECTED ╱
              │             ╲   ?    ╱
              │              ╲     ╱
              │                │ YES
              │                ▼
              │             ╱     ╲
              │   NO       ╱  HAS  ╲
              └──────────╱ CLOT BEEN╲
                         ╲ VERIFIED ╱
                          ╲   ?    ╱
                           ╲     ╱
                             │ YES
                             ▼
                    ┌─────────────────────┐
                    │   STOP TIMING  &    │
                    │ DISPLAY ELAPSED TIME│
                    └─────────────────────┘
```
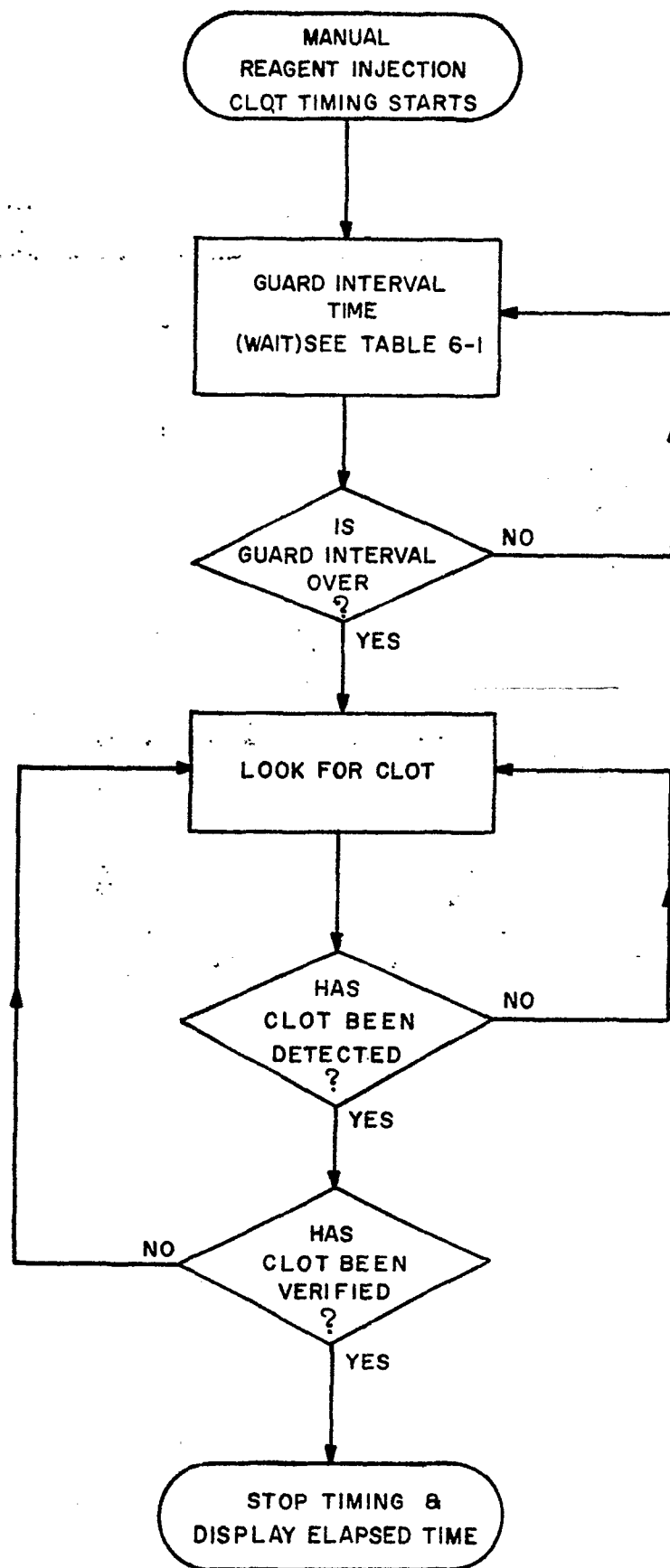
Figure 6-1.   E750 Operational Flow Diagram

TIMING
STARTS

TIMING
STOP

CLOT TIME

GUARD INTERVAL

CLOTTING ACTIVITY
OBSERVANCE

CLOT
VERIFIED

time ➤
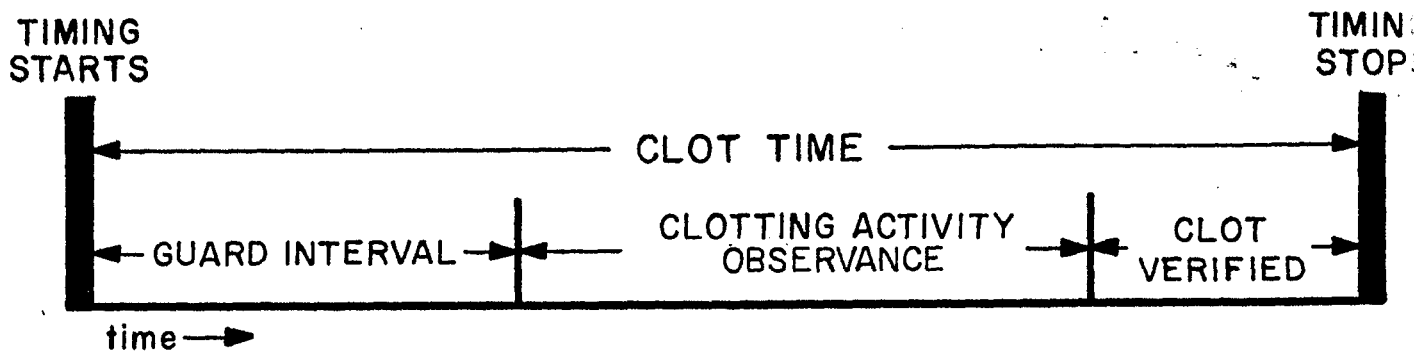
Figure 6-2.   E750 System Timing Diagram

750
REAGENT
DISPENSING
PIPETTE

START CLOT TIMING

STOP CLOT TIMING

| PHOTOLAMP 3 LEVELS OF INTENSITY | PHOTOCELL LIGHT SENSOR | OPTICAL DENSITY AMPLIFIER | CLOT TIMER | CLOT TIME DISPLAY |

TEST SPECIMEN

LIGHT BEAM

| INCUBATION HEATER | AC PRIMARY POWER CONTROL | POWER SUPPLIES |

AC INPUT FROM WALL OUTLET

Figure 6-3   Electra 750 Functional Block Diagram

TABLE 6-1   TABLE OF MODE CHARACTERISTICS
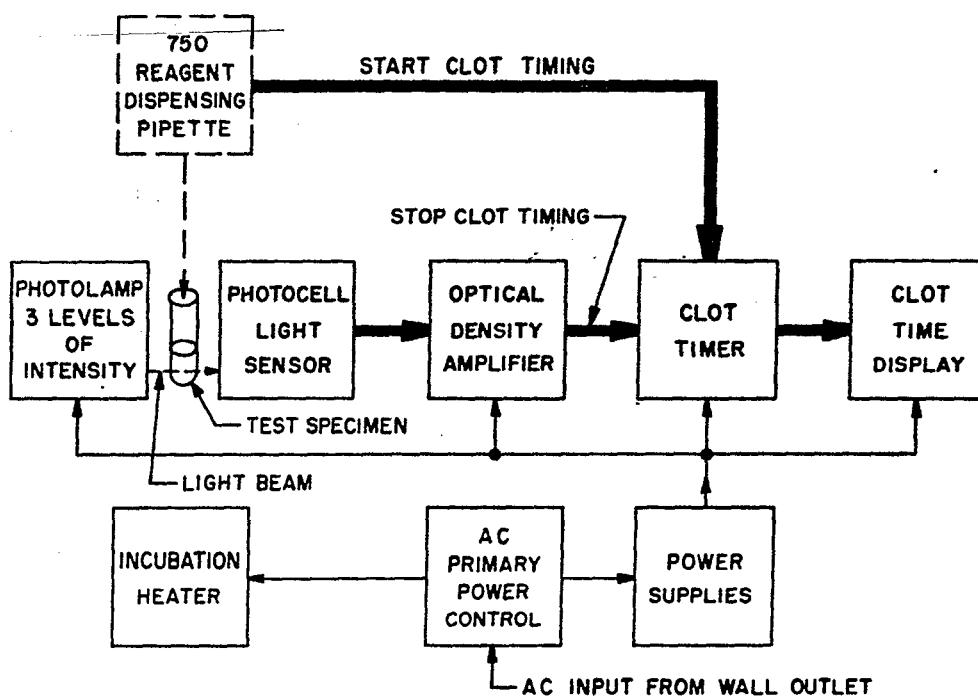
<u>MODE</u>

FUNCTION	PT	APTT	THROMBIN	SPEC 1	SPEC 2
Guard (seconds)	7	16	2	7	16
Acceptance Level (volts)	4.0	0.5	0.2	0.5	2.0
Tests Performed	PT	APTT	Thrombin Fibrinogen	Dilutions	APTT with Particulate Activator (G-D Auto APTT)

<u>Guard Interval</u> - Period of time prior to observation of the clot during which the mixing action and optical stabilization of the plasma and reagent mixture takes place. The instrument does not observe the activity in the tube during this period which appears as clotting activity to the instrument. The optical stabilization period is longer for APTT tests. Therefore, a longer guard interval is provided.

<u>Acceptance Level</u>-The level at which the clot signal indicates that a clot has developed. The lower the level, the more sensitive the instrument.

SECTION 7

COAGULATION

● HEMOSTASIS

INTRODUCTION

One of the major functions of blood is to maintain tissue stability, thereby keeping the internal environment of the body constant so that normal physiological processes may occur. In order to maintain this stability, blood must remain a fluid tissue within the confines of the circulatory system. The hemostatic mechanism is the mechanism by which normal blood flow and vascular integrity are maintained. For example, in the event that vascular integrity is compromised (i.e. a ruptured blood vessel) the hemostatic mechanism will act to restore that integrity and correct any interruption in circulation. However, hemostasis, by its broadest definition, does not just include the system which arrests bleeding. The definition must also include the system which lyses blood clots and the system of inhibitors which keep the first two systems from functioning in an inappropriate manner. These three systems form a delicately tuned mechanism of checks and balances which is called hemostasis. Hemostasis can be further examined from the aspect of its three major constituents -- vasculature, platelets, and plasma coagulation factors.

THE VASCULATURE

The vasculature is composed of arteries and arterioles, veins and venules, and capillaries. These components participate most significantly in two areas. First, when a blood vessel is traumatized, it responds by contracting. This contraction slows the flow of blood which allows the formation of the primary hemostatic plug (platelets) and subsequently the development of a stabilized fibrin clot (plasma coagulation factors). The second place where the vasculature participates in hemostasis is in the area of prevention of inappropriate clot formation. Normal vascular endothelium functions in a manner which discourages the formation of clots. When this normal endothelial layer is altered, the stage is set for the initiation of clot formation.

PLATELETS

In addition to these factors inherent in vessel function, the integrity of the hemostatic mechanism is also dependent upon the action of platelets.

To aid in hemostasis, platelets must not only be present in adequate numbers, but must function properly. For example, following injury to a vessel wall, platelets adhere to the exposed surface beneath the damaged endothelium. Circulating platelets stick to those already adhering and irreversible aggregation results.

Platelet aggregation serves two functions; the first is the
sealing of the ruptured vessel by forming a platelet plug and
the second is the release of several endogenous components. These
components act on the various parts of the hemostatic mechanism
(vasculature, platelets, coagulation factors) in a way that
potentiates the formation of blood clots. These components also
interact with other systems to cause a limitation of hemostasis
through a process of biomechanical feedback inhibition.

PLASMA COAGULATION FACTORS

At the time of this writing, there are fourteen substances that
are recognized as fitting into this category (see Appendix A).
These substances, called plasma coagulation factors, function
together in a "cascading" fashion, where each factor is changed
from an inactive "procoagulant" state to an active state by a
previously activated factor. Thus activated, it in turn activates
the next substance in the cascade. This cascade results in a
self-potentiating system of co-enzyme and enzyme reactions; the
end product of which is the formation of fibrin and a fibrin net.
Fibrin is the "cement" that stabilizes the primary hemostatic
(platelet) plug; and the fibrin net allows the addition of mass
to the clot by trapping blood components such as red blood cells.
In addition to the fourteen factors found in Appendix A, the
plasma coagulation factors can be considered to include the
naturally occurring circulating inhibitors of hemostasis such as
antithrombin III. Both the coagulant factors and the inhibitors
interact with the vasculature and the platelets to produce the
hemostatic response.

SUMMARY

To put all this in perspective: the normal hemostatic mechanism
is composed of a network of counterbalanced reactions which, when
considered as a whole, produce a self-potentiating and
self-limiting system capable of responding immediately to almost
any event which might cause the loss of blood. At the same time,
the resulting hemostasis will be limited to the area of bleeding
and should ultimately result in complete restoration of normal
blood flow. Finally, it can be seen that even a small abnormality
in this complex mechanism might have serious results. When a
clot forms in an intact blood vessel, the process is called
thrombosis. When no clot forms in a bleeding vessel, the result
is a hemorrhage. The function of plasma coagulation factors can
be tested by photo-optically monitoring the kinetics of fibrin
formation. The operation of the Electra Coagulation Timers is
based on this premise. Therefore, the following paragraphs will
emphasize related areas.

COAGULATION THEORY

THEORY OF BLOOD COAGULATION

The classic theory of blood coagulation states that blood remains
fluid in the absence of thromboplastin, which forms as a result

of tissue injury or cellular disintegration. Thromboplastin
participates in the conversion of prothrombin to thrombin with
subsequent fibrin formation.

PATHWAYS TO COAGULATION (Figure 7-1)

There are two types of thromboplastin involved in coagulation:
tissue thromboplastin (extrinsic pathway) and plasma thromboplastin
(intrinsic pathway). These can be distinguished by their
different reaction times; it takes longer to obtain a clot via
the intrinsic pathway than via the extrinsic pathway. Several
clotting factors participate in both the extrinsic and intrinsic
pathways to produce a prothrombin converting substance.

EXTRINSIC PATHWAY

The extrinsic pathway is triggered by tissue injury. When cells
are damaged, a tissue extract called tissue thromboplastin is
released. This extract along with the phospholipid material
Platelet Factor 3 (PF3), coagulation factor VII and calcium ions
(Ca^{++}) activates factor X to Xa. Factor Xa plus factor V, PF3
and Ca^{++} converts prothrombin to thrombin. Initiating thrombin
production by tissue thromboplastin and factor VII is called the
extrinsic pathway.

INTRINSIC PATHWAY

The intrinsic pathway reactions are triggered by many stimuli,
one of which is contact with abnormal surface. Two events that
occur very soon after initiation of the intrinsic pathway are
alteration of platelets and activation of factor XII. Platelet
alteration results in irreversible platelet aggregation and the
selective release of certain components, among them PF3.
Following the activation of factor XII, which complexes with
factor XI to form a contact activation product, clotting factors
IX, VIII, PF3, and Ca^{++} convert factor X to Xa. As in the
extrinsic pathway, factor Xa plus factor V, PF3 and Ca^{++}
converts prothrombin to thrombin. The conversion of prothrombin
to thrombin via the interaction of these clotting factors is
called the intrinsic pathway. There is a common sequence that
follows the conversion of factor X to Xa, and proceeds through
the formation of a stabilized clot.

STAGES OF COAGULATION

Since a universally accepted system of coagulation has not yet
been developed, one of the several hypothetical schemes is presented.
The coagulation factors are divided into three stages of activity.

STAGE 1:   GENERATION OF THROMBOPLASTIC ACTIVITY

The plasma thromboplastic activity necessary to convert
prothrombin is produced in Stage 1 through the interaction
of platelets with factors XII, XI, IX, and VIII (intrinsic
pathway) or through the release of tissue thromboplastin from
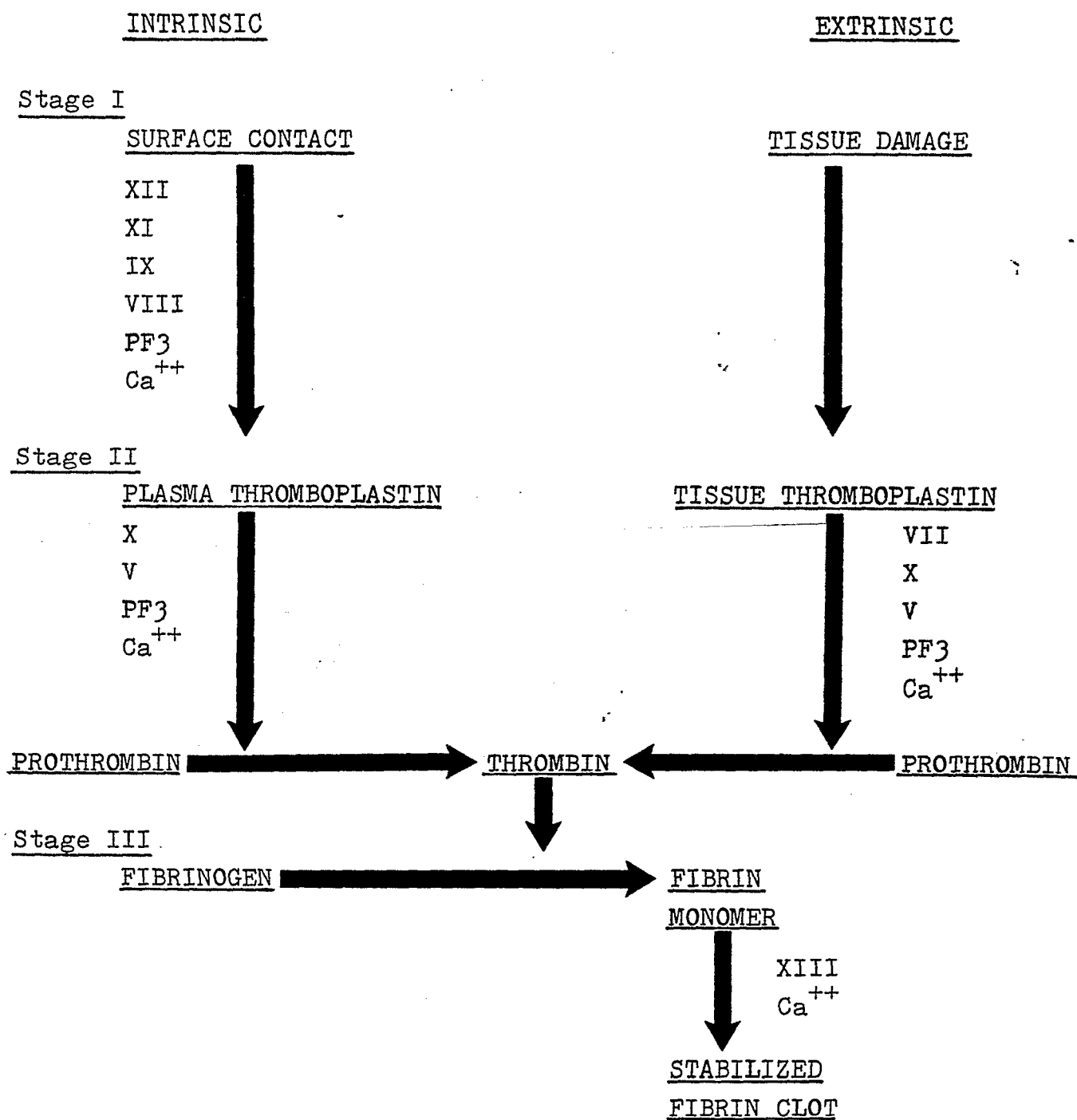
Figure 7-1 Pathways to Coagulation

injured tissues (extrinsic pathway). The activated partial
thromboplastin time test is the best choice for screening and
identification of Stage 1 deficiencies of the intrinsic
pathway.

STAGE 2:   GENERATION OF THROMBIN

The plasma thromboplastin (or tissue thromboplastin plus
factor VII) produced in Stage 1, in the presence of plasma
factors V and X, converts prothrombin to the active enzyme
thrombin. The one-stage prothrombin time test best detects
deficiencies occurring in Stage 2.

STAGE 3:   CONVERSION OF FIBRINOGEN

Thrombin converts fibrinogen (factor 1) to fibrin and a fibrin
clot is formed that is stabilized in the presence of factor XIII.
Thrombin time measures the concentration and activity of
fibrinogen in Stage 3.

● RELATED SYSTEMS

FIBRINOLYTIC SYSTEM

Plasma contains a second system called the fibrinolytic system.
This system is composed of the inactive precursor plasminogen,
plus activators, and inhibitors; and its pathways interconnect
with those of coagulation. Two functions attributed to the
fibrinolytic system in its normal response to injury are:

   1.   Restriction of fibrin formation to the injured area.
   2.   Clot dissolution through fibrin digestion.

INHIBITORY SYSTEM

Another system involved with plasma coagulation factors is
composed of naturally occurring inhibitors, such as antithrombin
III and rapid antiplasmin. These "anti-factors" act at many
different points in the hemostatic mechanism in order to limit
the other systems' functions to appropriate conditions.

SUMMARY

When all systems are operating properly, a normal hemostatic
response might be as follows:

   1.   Before any trauma, all aspects of the mechanism exist
        in a dynamic equilibrium state in which no blood
        clotting occurs.

   2.   When a blood vessel is cut, several things start to
        happen:

            a.   Tissue thromboplastin enters the blood
                 stream and activates the extrinsic pathway.

b. The exposed tissues at the sight of injury provide an abnormal surface for contact activation of the intrinsic pathway.

c. Platelets adhere to the injured area and more platelets cohere (aggregate). The platelets release PF3, Ca^{++} and substances which cause vasoconstriction. The resulting reduction of blood flow allows the consolidation of platelets, fibrin, and-other constituents into a clot.

d. The fibrinolytic system is activated by thrombin and other substances.

3. The circulating inhibitors limit the response to the immediate area of injury.

4. Gradually the tissue damage is repaired and the bulk of the clot is digested.

5. Normal equilibrium and flow are restored.

● BLEEDING DISORDERS

Bleeding disorders may be cuased by congenital or acquired anomalies involving one or more of the three mechanisms needed to maintain hemostasis; the vasculature, platelets and/or plasma coagulation factors. Some causes of bleeding disorders can be generally categorized as follows:

Defective Vascular Endothelium
Platelet Disorders
Congenital Clotting Factor Disorders
Acquired Clotting Factor Deficiencies

● IDENTIFICATION OF SPECIFIC CLOTTING FACTOR DISORDERS

Most congenital clotting factor disorders are the result of decreased or abnormal production of a single procoagulant. By using a partial thromboplastin time, a prothrombin time, a thrombin time and substitution studies, identification of the clotting factor disorder can be made.

ACTIVATED PARTIAL THROMBOPLASTIN TIME TEST (APTT)

This test is useful in the detection of latent bleeders. The APTT is prolonged in patients with deficiencies of clotting factors in the intrinsic pathway, namely, factors XII, XI, IX VIII, X, V, II (prothrombin) and I (fibrinogen). The APTT is also prolonged during administration of heparin, the coumarin derivatives, and in the presence of inhibitors other than those occurring normally.

PROTHROMBIN TIME TEST (PT)

Congenital clotting factor deficiencies of fibrinogen,
prothrombin, factors V, VII, or X can be detected with the PT.
Because the test detects deficiencies of five individual
clotting factors, it is a recommended adjunct to the APTT
test and is valuable as a diagnostic aid. Note that factors
I, II, V and X are common to both the intrinsic and extrinsic
pathways, and a deficiency of one or more of these factors
results in a prolongation of the activated partial thromboplastin
time and prothrombin time.

More frequently, the prothrombin time is prolonged because of
multiple clotting factor deficiencies as seen in severe liver
disease, vitamin K deficiency or the ingestion of coumarin type
medication. The prothrombin time may also be prolonged during
the administration of heparin.

THROMBIN TIME TEST

The Thrombin Time Test is used to evaluate the final phase of
blood coagulation. Thrombin is an enzyme that catalyzes the
conversion of fibrinogen to fibrin.

SUBSTITUTION STUDIES

When the APTT is abnormal and the PT is normal, the possible
factor deficiency lies in that part of the system involving the
intrinsic pathway. Substitution studies are necessary for
further identification

When the APTT is normal and the PT is abnormal, the factor
deficiency lies in that part of the system involving the
extrinsic pathway. No further testing is required because
factor VII is the only factor exclusive to the extrinsic
pathway.

When the APTT and PT are both abnormal, the thrombin time test
which is specific for fibrinogen is used to rule out the
prolongation being caused by a possible low fibrinogen, heparin
or other inhibitors. If the thrombin time is normal, the
problem is narrowed down to a factor deficiency other than
fibrinogen that is common to both pathways, namely, factor X,
V or II.

● ANTICOAGULANT THERAPY

Thrombosis is the term used to describe the closing of a blood
vessel by a clot that remains at its point of origin. A thrombus
may be a platelet aggregate (white thrombus) or a blood clot
(red thrombus). An embolus may be an aggregate or clot carried by
circulating blood from its point of origin, and forced into a
smaller vessel, thereby causing an obstruction. In either case,
anticoagulant therapy may be indicated.

LABORATORY TESTS

When laboratory tests are performed to evaluate each of the
major mechanisms involved in hemostasis, i.e. vasculature,
platelets and coagulation factors, a screening battery usually
includes the following:

> Bleeding Time
> Platelet Count
> Partial Thromboplastin Time
> Prothrombin Time
> Thrombin Time

BLEEDING TIME

The bleeding time evaluates hemostasis in the microcirculatory
system by platelets in primary response to injury, i.e., the
ability to form an effective platelet plug. The normal bleeding
time, depending upon the method used, is from 1 to 10 minutes.

PLATELET COUNT

The platelet count records the number of circulating platelets.
They must be present in adequate numbers and have proper function
to aid in hemostasis. The normal platelet count is approximately
150,000 to 450,000 per cubic millimeter of blood.

PARTIAL THROMBOPLASTIN TIME

The partial thromboplastin time measures the factors in the
intrinsic pathway. The test may be performed unactivated or
activated. Normal times for unactivated are in the range from
50 to 100 seconds; for activated 20 to 45 seconds. These ranges
will vary depending upon the particular test system used.

PROTHROMBIN TIME

The prothrombin time measures the factors in the extrinsic pathway.
Normal times are in the range from 10.0 to 13.0 seconds.

THROMBIN TIME

The thrombin time measures the transformation of plasma fibrinogen
to fibrin. Normal values are 200mg to 400mg/100ml of plasma.

The last three tests can be performed on the Electra.

COAGULATION TESTING

INTRODUCTION

This section describes operating protocols for performing
specific coagulation tests. Specific information on operation
of the Electra 750 is contained in Section 5, Operation. The
operator should have a thorough knowledge of the material in
Section 5 before proceeding in this section.

SPECIMEN COLLECTION

It is important that blood be collected with generally accepted
good practice in order to obtain valid coagulation results.
Collection should be accomplished by a clean venipuncture and
collected in a plastic syringe or siliconized glass tube. The
anticoagulant should be either 3.8% sodium citrate or 0.1 M
sodium oxalate in a ratio of 9 parts blood to 1 part
anticoagulant. EDTA and sodium heparin are generally not
acceptable anticoagulants for coagulation studies. After
collection, the blood specimens should be centrifuged as soon
as possible and the plasma removed from the cells. Recommended
centrifugation is 1000 RCF (g's) for 15 minutes, unless otherwise
noted. Specimens are to be kept cold while stored, and should
be tested as soon as possible. In general, all plasma should be
tested within 4 hours of being drawn.

● PREPARATION OF REAGENTS AND CONTROLS

BASIC RULES GOVERNING REAGENTS AND CONTROLS

a)   All reagents and controls should be reconstituted
     according to the manufacturer's instruction. Use
     only de-ionized, distilled water for reconstitution.

b)   Use clean glassware. Glassware should be cleaned
     in chromic acid solution.

c)   Reagents and controls should be reconstituted gently.
     When mixed, they should be rocked and never shaken
     unless otherwise noted.

d)   Reconstituted reagents should sit for at least 15
     minutes before using for test. Gently rock prior
     to using.

e)   Run controls in duplicate before and after each test
     run.

f)   Use the same lot number of reagent and control for all
     the tests. Record the lot numbers and expiration dates
     for all reagents and controls used.

PREPARATION OF CALCIUM CHLORIDE

MOLARITY	PROCEDURE
0.02 M	Weigh into a 100 ml volumetric flask 0.222 g anhydrous calcium chloride, dissolve and dilute to volume with distilled water.
0.025 M	Weigh into a 100 ml volumetric flask 0.277 g anhydrous calcium chloride, dissolve and dilute to volume with distilled water.
0.03 M	Weigh into a 100 ml volumetric flask 0.332 g anhydrous calcium chloride, dissolve and dilute to volume with distilled water.
0.10 M	Weigh into a 100 ml volumetric flask 1.110 g anhydrous calcium chloride, dissolve and dilute to volume with distilled water.

PLASMA AND SERUM PREPARATION

The following paragraphs describe various plasma and serum reagents used in coagulation testing. This list contains those items known to MLA at the time of printing, other products performing the same function may exist.

PLATELET RICH PLASMA

Centrifuge anticoagulated whole blood at 500 RCF (g's) for 5 minutes. Following centrifugation immediately remove the plasma. Keep the plasma on ice or refrigerate $4°-10°$ C until used.

PLATELET POOR PLASMA

Centrifuge anticoagulated whole blood at 1,000 RCF (g's) for 15 minutes. Following centrifugation, immediately remove the plasma. Keep the plasma on ice or refrigerate $4°-10°$ C until used.

ADSORBED PLASMA

Adsorbed normal plasma is a source of factors I, V, VIII, XI, and XII. It can be prepared as follows:

1. ALUMINUM HYDROXIDE (Al(OH)$_3$) ADSORBED PLASMA

   Pipette into a 13 X 100 mm test tube 1 ml of citrated plasma, add 0.1 ml aluminum hydroxide (Amphojel, Wyeth). Mix and incubate the mixture in a 37°C water bath for 3 minutes. Centrifuge the mixture at high speed for 10 minutes. Carefully remove the supernatant plasma with a Pasteur pipette. The supernatant plasma must have a prothrombin time greater than 60 seconds. Repeat adsorption is necessary if the prothrombin time is less than 60 seconds.

2. BARIUM SULFATE (BaSO$_4$) ADSORBED PLASMA

   Pipette into a 13 X 100 mm test tube 1 ml of oxalated plasma, add 100 mg barium sulfate. Place the plasma-barium sulfate mixture into a 37°C water bath for 15 minutes. Stir the mixture constantly. Centrifuge the mixture at high speed for 10 minutes. Carefully remove the supernatant plasma with a Pasteur pipette. The supernatant plasma must have a prothrombin time greater than 60 seconds. Repeat adsorption is necessary if the prothrombin time is less than 60 seconds.

AGED SERUM

Aged normal serum is a source of factors VII, IX, X, XI, and XII. Place freshly drawn blood into a glass test tube and allow the blood to clot. Place the tube into a 37°C water bath for 4 hours. Following the 4 hour incubation period, centrifuge and remove the serum. Place the serum into 37°C water bath for 24 hours. Divide the serum into 1.0 ml aliquots and store in a -20°C freezer.

OTHER REAGENTS

SODIUM CHLORIDE 0.85%

Weigh into a 100 ml volumetric flask 0.85 g sodium chloride, dissolve and dilute to volume with distilled water.

SODIUM CITRATE 3.8%

Weigh into a 100 ml volumetric flask 3.8 g of trisodium citrate (dihydrate), dissolve and dilute to volume with distilled water. Filter the solution and store in refrigerator.

SODIUM OXALATE 0.1 M

Weigh into a 100 ml volumetric flask 1.34 g sodium oxalate, dissolve and dilute to volume with distilled water. Filter the solution and store in refrigerator.

CITRATED SALINE

Add 1 part 3.8% sodium citrate to 5 parts 0.85% sodium chloride.

● COAGULATION TEST PROTOCOLS

The procedures presented below provide the specific protocol for performing the indicated test. These procedures have been tested in our lab, and provide precise, accurate and reproducible results. Good laboratory practice must exist to obtain good results.

Specific procedures for all clotting tests have not been provided due to the large number and variations of clotting tests. However, most tests are performed in a manner directly analagous to the common test procedures provided here. Additional material about these other tests may be obtained directly from MLA.

● PROTHROMBIN TIME (PT) TEST

PRINCIPLE

In this test, clotting is initiated by adding tissue thromboplastin and calcium to plasma. This test activates Stages 2 and 3 in the extrinsic clotting system. The Prothrombin Time Test is most commonly used to monitor patients on oral anti-coagulant therapy, because it is sensitive to factors II, VII, and X. Normal clot time is usually from 10 to 13 seconds. Patients on oral anti-coagulant therapy are usually medicated so that PT is 2 to $2\frac{1}{2}$ times normal.

MATERIAL REQUIRED

   Electra 750
   0.2 ml Instrument Pipette
   0.1 ml Pipette - MLA #1055
   Pipette Tips - MLA #9620
   Test Cuvettes - MLA #9005
   Stopwatch
   Test Tube Rack

REAGENTS REQUIRED

   Thromboplastin
   Normal Control Plasma or fresh normal pool

TEST PROCEDURE

a) Turn on Electra 750. Warm up instrument for 5 minutes or until AT TEMP lights, whichever is later.- Perform abbreviated O.C.T. See section 5.

b) Check that LAMP LEVEL switch is in "B" (middle position).

c) Reconstitute reagents and controls per manufacturer's instruction.

d) Pre-warm thromboplastin in REAGENT reservoir. Use magnetic stirring bar for reagents that require agitation (e.g. General Diagnostics Simplastin).

e) Set Mode Switch to PT.

f) Pipette 0.1 ml of control or patient plasma, in duplicate, into the bottom of MLA test cuvettes and place in rack.

g) Place first sample in incubation station #1 and start stopwatch.

h) After 30 seconds, place next sample in incubation station #2.

i) Continue in this manner adding next tube every 30 seconds.

j) After 180 seconds (3 minutes) take sample from incubation station #1 and place it in test station.

k) Using 0.2 ml (red top) instrument pipette, aspirate 0.2 ml of warm thromboplastin and align pipette over test station. Firmly push pipette plunger and hold it down for one second to start test.

l) When timer stops, record clot time.

m) Continue in this manner, adding new sample to heating block and then starting test every 30 seconds.

n) Use clean pipette tip on instrument pipette for each test to prevent carry-over contamination.

INTERPRETATION OF TEST RESULTS

The PT should be reported in conjunction with the normal range. The normal range varies depending upon the particular reagent and laboratory technique used. Therefore, a normal range must be determined for each laboratory.

Prolongation of the PT indicates an abnormality related to one of the clotting factors affecting the test or multiple factor deficiencies such as occur with the administration of coumarin type anticuagulants. For identification of specific factor deficiencies, Factor Substitution and Factor Assay test should be performed. Using good techniques with the PT test, the user can expect to achieve an average coefficient of variation of 0.75%.

PROTHROMBIN ACTIVITY ASSAY

PRINCIPLE

Oral anticoagulant therapy can be monitored by a Prothrombin Time test as a test for deficiencies in factors II, VII, and IX. Any prolongation of the time required to form fibrin indicates that one or more of these factors is present in less than normal concentration. The reduction of activity to 10-30 percent of normal activity is considered the therapeutic range.

A standard curve is prepared by obtaining clot times from saline dilutions. The times are plotted on graph paper with cartesian coordinates on which the prothrombin time in seconds is on the vertical axis and the percent dilution is on the horizontal axis (Figure 8-1). The construction of this curve permits interpretation of any prothrombin times obtained with the particular sample of thromboplastin which was used to develop the curve.

A new curve should be prepared with a change in reagent, change in lot number and periodically (six months). Checks of the system should be made on a daily basis, using both normal and abnormal controls.

MATERIAL REQUIRED

Electra 750
0.2 ml Instrument Pipette
0.1 ml Pipette - MLA #1055
0.5 ml Pipette
1.0 ml Pipette
Pipette Tips - MLA #9620
Test Cuvettes - MLA #9005
Stop watch
Graph Paper
Test Tube Rack

REAGENTS REQUIRED

Thromboplastin
Normal Control Plasma or fresh normal pool
0.85% Saline Solution

PREPARATION OF STANDARD DILUTIONS

Two common procedures used in the preparation of dilutions are

included here to provide (A) 100%, 50%, 25%, 12½%, and (B) 100% down to 10% in 10% increments.

PROCEDURE A - for 100%, 50%, 25% and 12½% test specimens. To obtain the above dilutions perform the following steps:

a) Place 0.1 ml of "normal" plasma in each of two cuvettes; these are your 100% samples.

b) Place 0.5 ml of "normal" plasma in a cuvette and add 0.5 ml of 0.85% saline solution. Mix well, but avoid excessive action that will foam specimen. This is your 50% specimen. Extract two 0.1 ml specimens for your test samples.

c) Place 0.5 ml of 50% specimen of step b) in fresh cuvette. Add 0.5 ml of 0.85% saline solution. Mix well, but avoid foaming. This is your 25% specimen. Extract two 0.1 ml specimens for your test samples.

d) Place 0.5 ml of 25% specimen prepared in step c) into fresh cuvette and add 0.5 ml of 0.85% saline solution. Mix well, but avoid foaming. This is your 12½% specimen. Extract two 0.1 ml specimens for your test samples.

PROCEDURE B - for 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% test specimens.

a) Take ten fresh cuvettes and place them in test tube rack. Place 1.0 ml of plasma into test tube #1, 0.9 ml of plasma into test tube #2, progressively reducing plasma by 0.1 ml until you have ten specimens ranging from 1.0 to 0.1 ml, as shown below.

TEST TUBE	1	2	3	4	5	6	7	8	9	10
PLASMA (ml)	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
SALINE (ml)	-	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
DILUTION	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%

b) Insert 0.1 ml of 0.85% saline solution into test tube #2, progressively increasing the saline solution by 0.1 ml until you have ten specimens ranging from 100% to 10%, as shown above.

c) Extract at least two 0.1 ml specimens from each of above dilutions as your test samples for running your Prothrombin Activity Curve.

TEST PROCEDURE - STANDARD CURVE

The Prothrombin Activity Curve is generated by performing a PT test in duplicate on each of the diluted samples. The clot times are then plotted on graph paper using cartesian coordinates as shown in Figure 8-1.

Follow the PT test procedure as given in section 8 with the
following exception. Set Mode Switch to SPEC. 1. In this
position the instrument has extra sensitivity and can therefore
detect clots with very low density.

TEST PROCEDURE - PATIENT PLASMA

Perform PT procedure in duplicate on patient plasma.

INTERPRETATION OF TEST RESULTS

The patient's clotting time is found on the vertical axis and
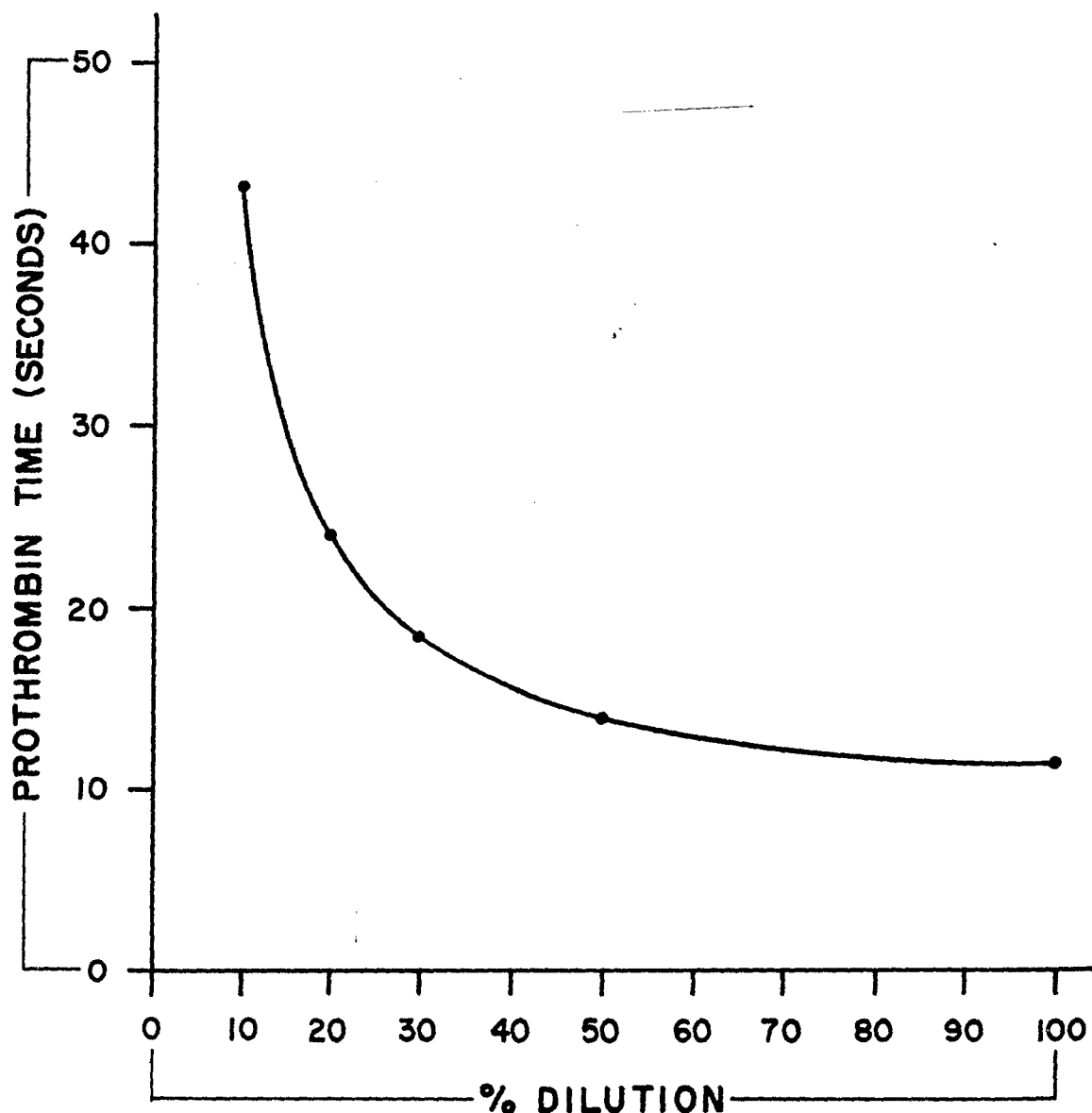the corresponding percent activity is read from the horizontal
axis.

Figure 8-1   Saline Dilution Curve

PT SUBSTITUTION TEST

PRINCIPLE

The Prothrombin Time test is sensitive to plasma clotting factors I, II, V, VII, and X. Prolongation of the Prothrombin Time can be caused by a decreased concentration of a specific procoagulant. Through the substitution of reagents containing specific procoagulants and known deficient plasmas, the identification of the deficient procoagulant is possible.

The Activated Partial Thromboplastin Time test and Fibrinogen Assay should be performed on all plasmas studied with the Substitution Test. The Prothrombin Time, the Activated Partial Thromboplastin Time, and Fibrinogen Assay are necessary for identification of the clotting factor deficiency.

MATERIAL REQUIRED

Electra 750
0.2 ml Instrument Pipette
0.1 ml Pipette - MLA #1055
Pipette Tips - MLA #9620
Test Cuvettes - MLA #9005
Stopwatch
Test Tube Rack

REAGENTS REQUIRED

Thromboplastin
Normal Control Plasma or a fresh normal pool
Adsorbed Plasma
Aged Normal Serum
APTT Reagent
Ribrinogen Assay Reagents
Calcium Chloride (Consult APTT Reagent manufacturer's literature)

TEST PROCEDURE

a) Perform PT test.

b) Perform APTT test.

NOTE

An abnormal PT accompanied by a normal APTT indicates a factor VII deficiency. No further testing is necessary for identification of the deficient factor.

c) Perform Fibrinogen Assay.

d) Just before use, prepare a 0.1 ml + 0.1 ml mixture of normal plasma and unknown plasma.

e) Perform PT test on this 0.2 ml mixture.

f) Repeat Steps d) and e) using aged serum and unknown plasma, then adsorbed plasma and unknown plasma.

INTERPRETATION OF TEST RESULTS

Normal plasma is presumed to contain a normal level of all coagulation factors. When the test plasma's PT is prolonged compared to the normal value, but it is corrected significantly toward the normal value by the Substitution Test, then a factor deficiency has been established.

The identity of the specific factor deficiency can be determined from Table 8-1. Also see Appendix B.

TABLE 8-1. IDENTIFICATION OF FACTOR DEFICIENCY BY PT SUBSTITUTION TEST

PT SUBSTITUTION TEST	APTT	FACTOR DEFICIENCY
Corrected by Adsorbed Plasma	Prolonged	Factor V
Corrected by Aged Serum	Prolonged	Factor X

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) TEST

PRINCIPLE

The Activated Partial Thromboplastin Time test is a screening test for the intrinsic coagulation system. This test is sensitive to all the coagulation factors, with the exception of factors VII, and XIII. A reagent is added to the plasma which takes the place of the platelets in the coagulation mechanism. This reagent also contains a substance which activates factors XII and XI. The activation time and temperature must be well controlled for proper activation to take place. Calcium is then added to trigger the coagulation, by providing the ions required.

The normal clot time for the Activated Partial Thromboplastin Time varies considerably with the particular reagent used. In general, the normal time is from 24 to 45 seconds.

MATERIAL REQUIRED

    Electra 750
    0.1 ml Instrument Pipette
    0.1 ml Pipette - MLA #1055
    Pipette Tips - MLA #9620
    Test Cuvettes - MLA #9005
    Stopwatch
    Test Tube Rack

n) Use clean pipette tip on instrument pipette for each tes
to prevent carry-over contamination.

<u>NOTE</u>

Precise control of incubation time is
essential for good results in the APTT
test. Significant deviations from the
specified activation time will reduce
the reproducibility of the test.

INTERPRETATION OF TEST RESULTS

The APTT should be reported in conjunction with the normal rang
The normal range varies considerably depending upon the particu
reagent and laboratory techniques used. Therefore, a normal ra
must be determined for each laboratory.

Prolongation of the APTT indicates an abnormality related to on
or more of the clotting factors affecting this test or the
presence of anticoagulants such as heparin. For identification
of particular coagulation factor deficiencies, Factor
Substitution and Assay tests should be performed. Using good
techniques with the APTT test, the user can expect to achieve
an average coefficient of variation of 1.3%

● APTT SUBSTITUTION TEST

PRINCIPLE

The Activated Partial Thromboplastin Time is essentially a
control test for all clotting factors, except Factors VII and XII
A prolongation of the Activated Partial Thromboplastin Time is
usually caused by a decrease in concentration of a specific
procoagulant. Identification of a deficient procoagulant is
possible through a substitution of reagents containing specific
procoagulants and known deficient plasmas.

A Prothrombin Time and Fibrinogen Assay should be performed on all
plasmas studied with the Substitution Test. The Activated Partial
Thromboplastin Time, the Prothrombin Time, and Fibrinogen Assay
are necessary for identification of the deficient coagulation
factor.

MATERIALS REQUIRED

Electra 750
0.1 ml Instrument Pipette
0.1 ml Pipette - MLA #1055
Pipette Tips - MLA #9620
Test Cuvettes - MLA #9005
Stopwatch
Test Tube Rack

REAGENTS REQUIRED

APTT Reagent
Calcium Chloride (consult APTT Reagent manufacturer's
literature)
Normal Control Plasma or fresh normal pool

TEST PROCEDURE

a) Turn on Electra 750. Warm up instrument for 5 minutes or
   until AT TEMP lights, whichever is later. Perform the
   abbreviated O.C.T.

b) Check that lamp level switch is in "B" (middle position).

c) Reconstitute reagents and controls per manufacturer's
   instructions.

d) Prewarm Calcium Chloride in calcium reservoir.

e) For ellagic acid type reagent, set mode switch to APTT.
   For particulate type activator materials, set mode switch
   to SPEC 2.

NOTE

At time of printing, common ellagic acid
activators are Dade Cephaloplastin or Actin,
or Ortho Thrombofax. A common type of
particulate activator is General Diagnostic's
Platelin Plus, or Automated APTT Reagent.

f) Pipette 0.1 ml of control or patient plasma, in duplicate,
   into the bottom of MLA test cuvettes, and place in rack.

g) Add to the first tube, 0.1 ml of APTT Reagent shake gently
   and place in incubation station #1, then start stopwatch.

h) After 100 seconds, add 0.1 ml APTT Reagent to the next
   cuvette with plasma. Shake gently and place in incubation
   station #2.

i) Continue in this manner, adding tube every 100 seconds.

j) After 300 seconds (5 minutes) take sample from incubation
   station #1 and place it in test station.

k) Using 0.1 ml (blue top) instrument pipette, aspirate 0.1 ml
   of calcium and align pipette over test station. Firmly
   push pipette plunger and hold it down for one second to
   start the test.

l) When timer stops, record clot time.

m) Continue in this manner, adding new sample to heating block,
   and then starting test every 100 seconds.

n) Use clean pipette tip on instrument pipette for each test
to prevent carry-over contamination.

NOTE

Precise control of incubation time is
essential for good results in the APTT
test. Significant deviations from the
specified activation time will reduce
the reproducibility of the test.

INTERPRETATION OF TEST RESULTS

The APTT should be reported in conjunction with the normal range.
The normal range varies considerably depending upon the particular
reagent and laboratory techniques used. Therefore, a normal range
must be determined for each laboratory.

Prolongation of the APTT indicates an abnormality related to one
or more of the clotting factors affecting this test or the
presence of anticoagulants such as heparin. For identification
of particular coagulation factor deficiencies, Factor
Substitution and Assay tests should be performed. Using good
techniques with the APTT test, the user can expect to achieve
an average coefficient of variation of 1.3%

●APTT SUBSTITUTION TEST

PRINCIPLE

The Activated Partial Thromboplastin Time is essentially a
control test for all clotting factors, except Factors VII and XIII.
A prolongation of the Activated Partial Thromboplastin Time is
usually caused by a decrease in concentration of a specific
procoagulant. Identification of a deficient procoagulant is
possible through a substitution of reagents containing specific
procoagulants and known deficient plasmas.

A Prothrombin Time and Fibrinogen Assay should be performed on all
plasmas studied with the Substitution Test. The Activated Partial
Thromboplastin Time, the Prothrombin Time, and Fibrinogen Assay
are necessary for identification of the deficient coagulation
factor.

MATERIALS REQUIRED

Electra 750
0.1 ml Instrument Pipette
0.1 ml Pipette - MLA #1055
Pipette Tips - MLA #9620
Test Cuvettes - MLA #9005
Stopwatch
Test Tube Rack

REAGENTS REQUIRED

APTT Reagent
Calcium Chloride (Consult APTT Reagent manufacturer's literature)
Adsorbed Normal Plasma
Aged Normal Serum
Normal Control Plasma or fresh normal pool
Thromboplastin
Fibrinogen Assay Reagents

TEST PROCEDURE

a) Perform APTT test.

b) Perform PT test.

c) Perform Fibrinogen Assay.

d) Just before use, prepare a 0.1 ml + 0.1 ml mixture of normal plasma and unknown plasma.

e) Perform APTT test on this 0.2 ml mixture.

f) Repeat steps d) and e) using aged serum and unknown plasma, the adsorbed plasma and unknown plasma.

INTERPRETATION OF TEST RESULTS

Normal plasma is presumed to contain a normal level of all clotting factors. When the unknown plasma's APTT is prolonged compared to the normal value, but is corrected significantly toward the normal value by the Substitution Test, then a factor deficiency has been established.

The identity of the specific factor deficiency can be determined from Table 8-2.

TABLE 8-2

IDENTIFICATION OF FACTOR DEFICIENCY BY APTT SUBSTITUTION

APTT SUBSTITUTION TEST	PROTHROMBIN TIME	FACTOR DEFICIENT
Corrected by Adsorbed Plasma	Prolonged	V
Corrected by Adsorbed Plasma	Normal	VIII
Corrected by Aged Serum	Normal	IX
Corrected by Aged Serum	Prolonged	X
Corrected by Adsorbed Plasma and Aged Serum	Normal	XI and/or XII

FACTOR VIII AND IX ASSAYS

PRINCIPLE

The level of factor VIII or factor IX is determined by a substitution assay test, which measures the corrective effect on the APTT obtained when the test plasma is added to a specific factor deficient substrate plasma. By comparing the results obtained with the test plasma to results produced by known concentrations of factor VIII or factor IX, the percentage of the factor present can be determined. Normal plasma is considered to give 100% correction.

To obtain accurate, reproducible results, use a standard APTT contact activation time. A new calibration curve must be made when there is a change in the lot number of reagents and/or a change in collection system.

Factor VIII and factor IX assays are performed in exactly the same manner, except the corresponding factor deficient substrate is used.

MATERIALS REQUIRED

    Electra 750
    0.1 ml Instrument Pipette
    0.1 ml Pipette - MLA #1055
    0.5 ml Pipette
    1.0 ml Pipette
    Pipette Tips - MLA #9620
    Test Cuvettes - MLA #9005
    Stopwatch
    Test Tube Rack
    2 Cycle Log-Log Graph Paper

REAGENTS REQUIRED

APTT Reagent
Calcium Chloride (consult APTT Reagent manufacturer's
literature)
0.85% Buffered Saline Solution
Normal Control Plasma or fresh normal pool (citrate collection
is recommended)
Factor Deficient Substrate Plasma

NOTE

Specimens collected for this test must be
tested within 2 hours of being drawn.

PREPARATION OF STANDARD DILUTIONS

a) Pipette 0.8 ml of buffered saline into tube #1 and 0.5 ml
of buffered saline into tubes #2 through #7.

b) Pipette 0.2 ml of normal plasma into the first tube and mix
well.

c) Transfer 0.5 ml from tube #1 into tube #2 and mix well.
Continue through tube #7 as shown in the example.

EXAMPLE;

| TUBE	1	2	3	4	5	6	7
BUFFERED SALINE (ml)	0.8	0.5	0.5	0.5	0.5	0.5	0.5
NORMAL PLASMA (ml)	0.2	–	–	–	–	–	–
MIX AND TRANSFER (ml)		0.5	0.5	0.5	0.5	0.5	0.5
DILUTION	1:5	1:10	1:20	1:40	1:80	1:160	1:320
% OF FACTOR PRESENT	100	50	25	12.5	6.25	3.13	1.5

TEST PROCEDURE - STANDARD CURVE

a) Prepare sufficient quantity of factor deficient plasma and
normal pool to run the required tests.

b) Dispense into each of two cuvettes, 0.1 ml of 1:5 dilution,
0.1 ml of factor deficient plasma and place in rack.

c) Repeat for each level of dilution.
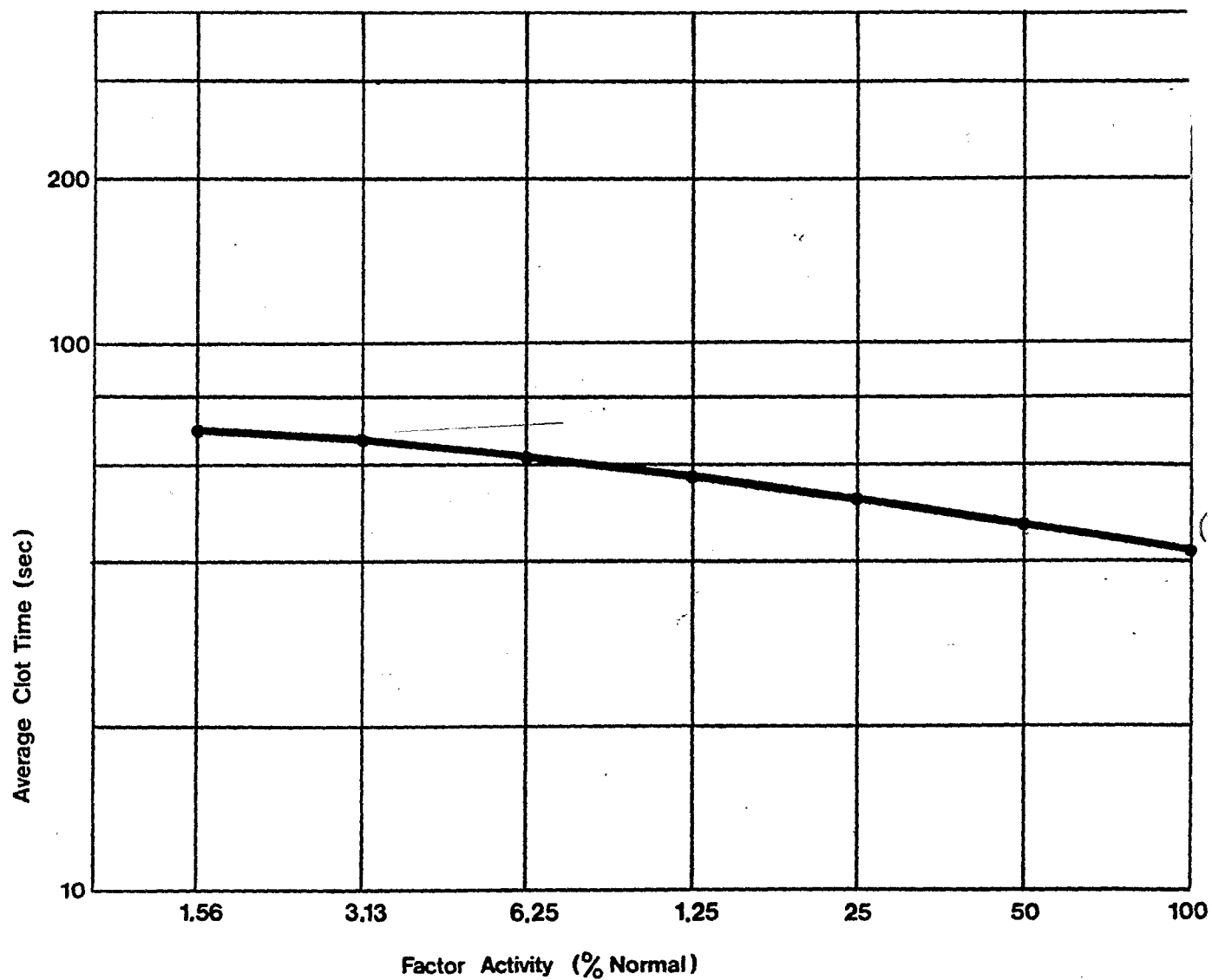
8-2 Typical Factor VIII Assay
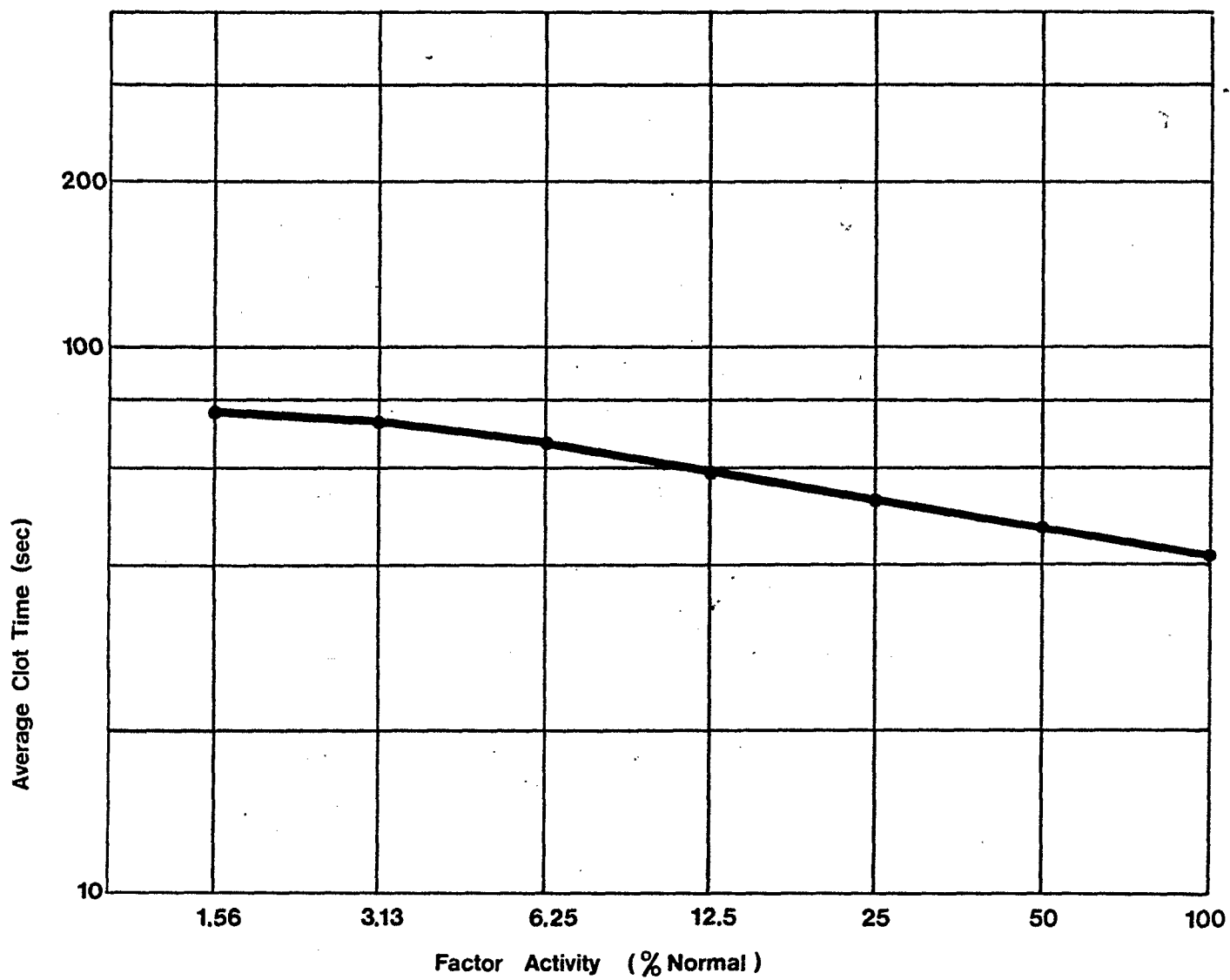
8-3 Typical Factor IX Assay Curve

d) Perform APTT test on 0.2 ml mixtures.

e) Average duplicate results, and plot on 2 cycle log-log graph paper with percent of dilution on horizontal axis, and time in seconds on vertical axis. Connected points should approximate a straight line. (See typical curves in Figures 8-2 and 8-3).

UNKNOWN PLASMA

a) To test unknown plasma, make 1:5 dilution of plasma using buffered saline.

b) Dispense into cuvette 0.1 ml of this plasma dilution, 0.1 ml of the factor deficient substrate plasma, and place in rack.

c) Perform APTT test in duplicate on this 0.2 ml mixture.

d) Average clot times from duplicate samples.

INTERPRETATION OF TEST RESULTS

To determine the amount of factor in the unknown sample, find the clot time of the sample on the vertical axis of the calibration curve. Read the percent factor activity off the horizontal axis. Report this percent activity. 50-150% is generally considered normal.

● HEPARIN ASSAY USING THE APTT

PRINCIPLE

The APTT test has been used for monitoring heparin therapy. Heparin inhibits the clotting mechanism at several stages and the APTT will reflect this effect. By diluting plasma with heparin, an in-vitro response curve relating the APTT to heparin concentration may be obtained. This curve is plotted as shown in Figure 8-4. The APTT of a patient may then be related to the heparin concentration by using this curve.

The heparin used should be identical to that administered to the patient. The plasma used to generate this curve should be a pool of fresh normal plasmas. This curve can be used to indicate how an "average" plasma responds, but individual responses can vary widely. Lyophilized control plasmas do not provide good results in this test.

Check of the system should be made daily, using both normal and abnormal controls.

CAUTION

The following cautions should be observed
to obtain the best possible results in
monitoring heparin therapy.

a) Results of heparin monitoring can be altered by the type of
anticoagulant used in the collection system. According to
studies, plasma drawn in citrate shows a greater sensitivity
to heparin than plasma drawn in oxalate.

b) An APTT should be run on the patient prior to heparin therapy
to establish base line data. Response curves must be
constructed using heparin employed in the therapy to
eliminate variables that may exist between heparins of
different manufacturers.

c) Testing of all samples must be performed within the same time
frame to prevent prolonged APTT determinations. Heparinized
plasma deteriorates more rapidly and is more sensitive to
variations in technique than plasma that does not contain
heparin.

d) Consideration must be given to the time of collection
because in vivo half life of heparin is approximately
1.5 hours. This effect is demonstrated by the immediate
anticoagulant action, with the degree decreasing rapidly
with time. When intermittent single intravenous
injections are administered, this action is most apparent.

e) Specimens should be collected with a minimum of trauma to
prevent releasing platelet factor 4, a heparin neutralizing
factor. Best lab practice dictates the use of plastic
syringes that contain the proper amount of anticoagulant.

f) Curves should be reestablished when lot number of reagent,
heparin manufacturer, or collection system changes.

MATERIAL REQUIRED

Electra 750
0.1 ml Instrument Pipette
0.1 ml Pipette - MLA #1055
Pipette Tips - MLA #9620
Test Cuvettes - MLA #9005
Stopwatch
Test Tube Rack
Semi-Log Graph Paper

REAGENTS REQUIRED

APTT Reagent
Calcium Chloride (consult APTT Reagent manufacturer's
literature)
Fresh Normal Pool or patient plasma, prior to heparin therapy.
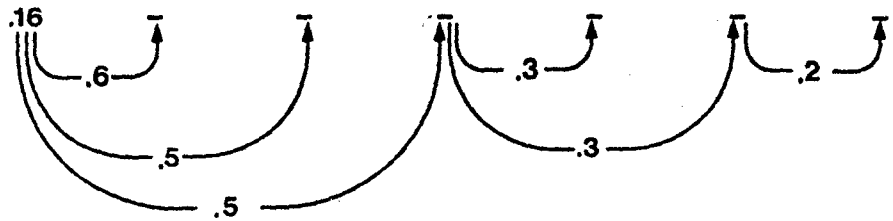Heparin used in patient therapy
0.85% Saline Solution

PREPARATION OF HEPARIN STANDARDS

NOTE

Heparin is generally available in 1000 units/ml
injectable doses. This type requires a 1:100
dilution with 0.85% saline for preparation of
a stock working heparin solution of 10 units/ml.
The heparin label should be consulted for
proper dilution.

a) Using normal pool plasma or patient plasma before therapy,
   prepare standards in the following concentrations - 0.8,
   0.6, 0.5, 0.4, 0.3, 0.2, 0.1 units/ml as follows:

Tube	1	2	3	4	5	6	7
Plasma (ml)	1.84	0.2	0.3	0.5	0.1	0.3	0.2
Heparin (10 U/ml)	.16						
Heparin Concentration	0.8	0.6	0.5	0.4	0.3	0.2	0.1

Mix & Transfer → .6   .5   .5   .3   .3   .2

b) Place seven tubes in test tube rack.

c) Add 1.84 ml normal plasma to test tube #1.

d) Add 0.16 ml of heparin solution to test tube #1 and shake
   gently. This is 0.8 units/ml solution.

e) Using pipette, aspirate 0.6 ml of solution from tube #1
   and dispense into tube #2.

f) Add 0.2 ml of normal plasma to tube #2 and shake gently.
   This is 0.6 units/ml solution.

g) Using pipette, aspirate 0.5 ml of solution from tube #1
   and dispense into tube #3.

h) Add 0.3 ml of normal plasma to tube #3 and shake gently. This is 0.5 units/ml solution.

i) Using pipette, aspirate 0.5 ml of solution from tube #1 and dispense into tube #4.

j) Add 0.5 ml of normal plasma to tube #4 and shake gently. This is 0.4 units/ml solution.

k) Using pipette, aspirate 0.3 ml of solution from tube #4 and dispense into tube #5.

l) Add 0.1 ml of normal plasma to tube #5 and shake gently, This is 0.3 units/ml solution.

m) Using pipette, aspirate 0.3 ml of solution from tube #4 and dispense into tube #6.

n) Add 0.3 ml of normal plasma to tube #6 and shake gently. This is 0.2 units/ml solution.

o) Using pipette, aspirate 0.2 ml of solution from tube #6 and dispense into tube #7.

p) Add 0.2 ml of normal plasma to tube #7 and shake gently. This is 0.1 units/ml solution.

TEST PROCEDURE - STANDARD CURVE

a) Perform APTT procedure in duplicate on each sample.

b) Using semi-log graph paper, plot APTT for each specimen on vertical (log) axis and concentration on horizontal (linear) axis. Connected points should approximate a straight line.

TEST PROCEDURE - PATIENT

a) Perform APTT procedure in duplicate on heparinized patient plasma.

INTERPRETATION OF TEST RESULTS

The heparin concentration of the patient plasma is determined by locating the patient's clot time on the vertical (log) axis of the heparin assay curve and then reading the corresponding heparin concentration from the horizontal (linear) axis.
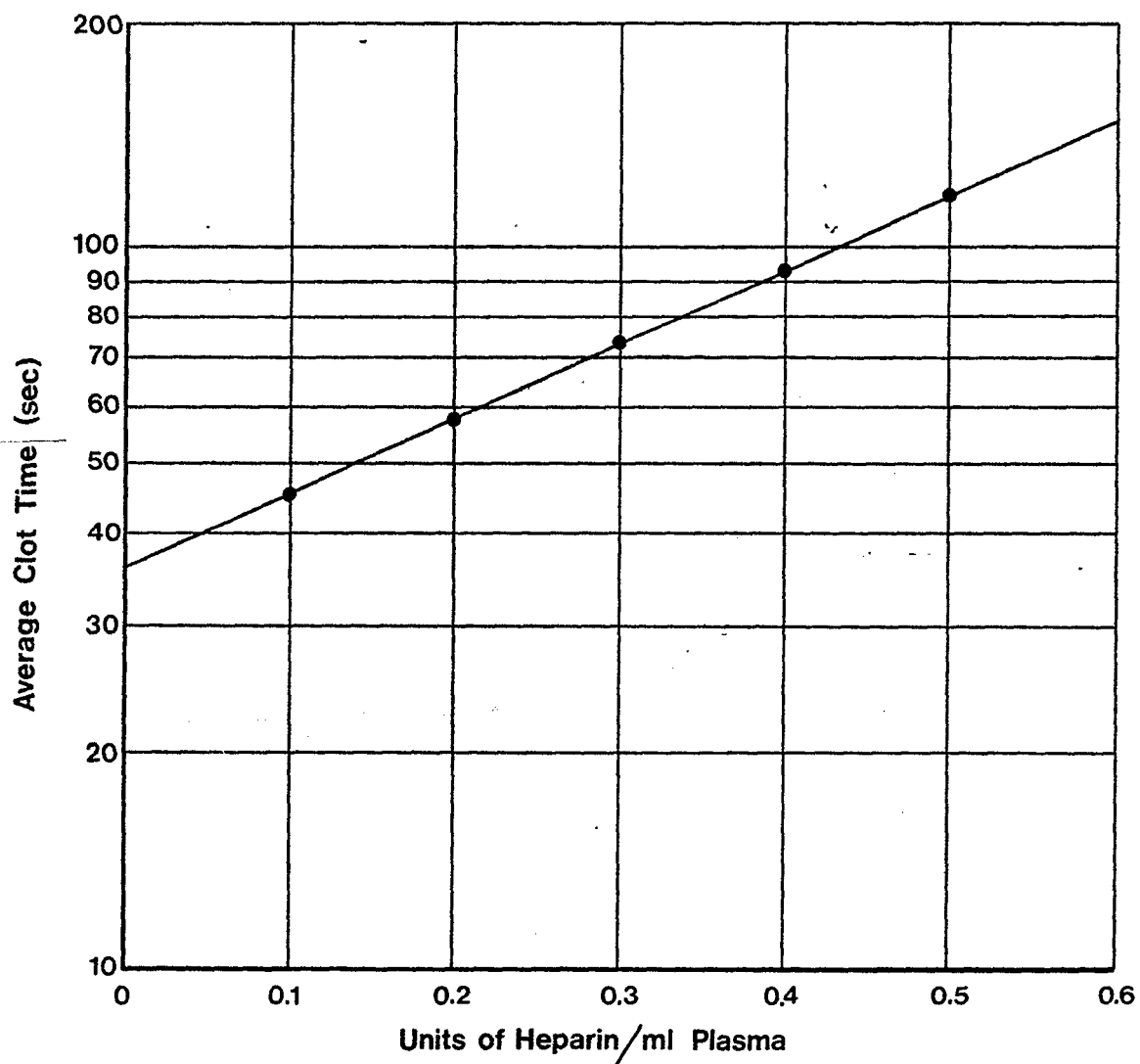
8-4   TYPICAL HEPARIN RESPONSE CURVE

QUANTITATIVE FIBRINOGEN ASSAY USING DADE FIBRINOGEN DETERMINATION KIT

PRINCIPLE

The thrombin clotting time of dilute plasma is inversely proportional to the fibrinogen concentration of the plasma, provided the thrombin concentration is high and the fibrinogen concentration is fairly low (5-80mg/dl). In this test, the amount of fibrinogen in a plasma sample is determined by comparing its thrombin clotting time to that of a standard plasma of known fibrinogen content.

The Electra 750 is capable of detecting fibrinogen concentrations as low as 10-14mg/dl. Normal concentrations in plasma are from 200-400mg/dl.

This procedure is specifically written to be used with the Dade Fibrinogen Determination Kit. While other kits are available, at the time of printing this is the most widely used kit. Procedures for other kits are similar.

MATERIALS REQUIRED

Electra 750
0.1 ml Instrument Pipette
0.2 ml Pipette - MLA #1056
0.5 ml Pipette
1.0 ml Pipette
Pipette Tips - MLA #9620
Test Cuvettes - MLA #9005
2 Cycle Log-Log Graph Paper - MLA #9041 or equivalent
Stopwatch
Test Tube Rack

REAGENTS REQUIRED

Dade Thrombin Reagent
Dade Fibrinogen Calibration Reference
Owren's Veronal Buffer

NOTE

These reagents are available individually or in kit form.

CAUTION

This procedure can only be performed on citrated plasma.

PREPARATION OF STANDARD DILUTIONS BASED ON ASSAY VALUE

Check the assay value of the Fibrinogen Calibration Reference. Perform the dilution procedure indicated in Table 8-3.

TABLE 8-3

DILUTIONS FOR RANGES OF FIBRINOGEN ASSAY VALUES

FIBRINOGEN CALIBRATION REFERENCE

ASSAY VALUE	PROCEDURE	DILUTIONS
210 - 280	A	1/5, 1/10, 1/15
280 - 350	B	1/5, 1/10, 1/20
350 - 450	C	1/10, 1/20, 1/25

PROCEDURE A

Preparation of Fibrinogen Standard Dilutions - 1/5, 1/10, 1/15.

a) Place 1.6 ml of Owren's Veronal Buffer into a suitable cuvette.

b) Add 0.4 ml of Fibrinogen Calibration Reference. Mix thoroughly, taking care not to cause foaming of sample. This cuvette contains 1/5 dilution.

c) Extract 0.5 ml of 1/5 specimen prepared in step b) above, and place in second cuvette.

d) Add 0.5 ml Owren's Veronal Buffer to second cuvette and mix thoroughly, avoid foaming. This second cuvette contains 1/10 dilution.

e) Extract 0.4 ml of 1/5 specimen prepared in step b) above, and place in third cuvette.

f) Add 0.8 ml of Owren's Veronal Buffer to third cuvette and mix thoroughly, avoid foaming. This third cuvette contains the 1/15 dilution.

PROCEDURE B

Preparation of Fibrinogen Standard Dilutions - 1/5, 1/10, 1/20.

a) Place 1.6 ml of Owren's Veronal Buffer into a suitable cuvette.

b) Add 0.4 ml of Fibrinogen Calibration Reference. Mix thoroughly, taking care not to cause foaming of the sample. This cuvette contains the 1/5 dilution.

c) Extract 0.5 ml of the specimen prepared in step b) above, and place in second cuvette.

d) Add 0.5 ml of Owren's Veronal Buffer to second cuvette and mix thoroughly, avoid foaming. This second cuvette contains 1/10 dilution.

e) Extract 0.4 ml of 1/5 specimen prepared in step b) above, and place in third cuvette.

f) Add 1.2 ml of Owren's Veronal Buffer to third cuvette and mix thoroughly, avoid foaming. This third cuvette conains 1/20 dilution.

PROCEDURE C

Preparation of the Fibrinogen Standard Dilutions - 1/10, 1/20, 1/25.

a) Place 1.8 ml Owren's Veronal Buffer into suitable cuvette.

b) Add 0.2 ml of Fibrinogen Calibration Reference. Mix thoroughly, taking care not to cause foaming of sample. This cuvette contains 1/10 dilution.

c) Extract 0.5 ml of the 1/10 specimen prepared in step b) above, and place in second cuvette.

d) Add 0.5 ml of Owren's Veronal Buffer to second cuvette and mix thoroughly, avoid foaming. This second cuvette contains 1/20 dilution.

e) Extract 0.4 ml of 1/10 specimen prepared in step b) above, and place in third cuvette.

f) Add 0.6 ml of Owren's Veronal Buffer to third cuvette and mix thoroughly avoid foaming. This third cuvette contains 1/25 dilution.

TEST PROCEDURE - STANDARD CURVE

NOTE

The standard curve must be repeated whenever a different lot number of Thrombin Reagent is used. It should also be done periodically to insure no changes in the curve take place.

a) Reconstitute Fibrinogen Calibration Reference and prepare dilutions in accordance with Table 8-3 and the appropriate procedure.

b) Reconstitute Thrombin Reagent

CAUTION

DO NOT WARM THROMBIN REAGENT

c) Turn on Electra 750. Warm up instrument for 5 minutes or until AT TEMP lights, whichever is later. Perform abbreviated O.C.T. See section 5.

d) Set Mode Switch to Thrombin and Lamp Level to B.

e) Pipette 0.2 ml of plasma into cuvettes.

NOTE

Run all tests in triplicate.

f) Place first sample in incubation station #1 and start stopwatch.

g) After 30 seconds, place next sample in incubation station #2 and continue in this manner adding next tube every 30 seconds.

h) After 180 seconds (3 minutes), place sample from incubation station #1 into test well.

i) Using 0.1 ml (blue top) instrument pipette, aspirate 0.1 ml of Thrombin Reagent and align pipette over test station. Firmly push plunger and hold down for one second to start test.

j) When timer stops, record clot time.

k) Continue in this manner, adding new sample to heating block and then starting test every 30 seconds.

l) Use clean pipette tip on instrument pipette for each test to prevent carry-over contamination.

PLOTTING STANDARD CURVE

The Fibrinogen Standard Curve is plotted on log-log graph paper. The graph paper provided with the kit may be used. However, the graph paper supplied by MLA is better suited for the Electra 750.

Plot the Standard Curve by performing the following steps:

a) Average triplicate clot times obtained for each dilution level.

b) Determine equivalent fibrinogen values for each dilution as follows: The assay value of the Fibrinogen Reference Plasma is assigned to the 1:10 dilution. The values of the dilutions are determined by multiplying the assay value by the factor given in Table 8-4.

TABLE 8-4

DILUTION MULTIPLYING FACTOR FOR FIBRINOGEN CURVE

DILUTION	FACTOR
1:5	2
1:10	1
1:15	0.67
1:20	0.5
1:25	0.4

c) Plot fibrinogen level versus average time, using the vertical axis for time and the horizontal axis for fibrinogen level.

d) The connected points should approximate a straight line (see Figure 8-5).

EXAMPLE:

Fibrinogen Reference FS-15 has an assay value of 229 mg/dl. Plasma is diluted in accordance with Table 8-3. In this case, the assay value requires dilutions of 1:5, 1:10 and 1:15. Using Table 8-4, the fibrinogen level is as follows:

DILUTION	MULTIPLY	FIBRINOGEN VALUE
1:5	2	2 X 229 = 458
1:10	1	1 X 229 = 229
1:15	0.67	0.67 X 229 = 153

These values are then plotted with the respective clot times to permit construction of the curve shown in Figure 8-5.

TEST PROCEDURE - PATIENT PLASMA

a) A control plasma should be run to check the system if a previously determined standard curve is being used. Dilute plasma 1:10 in Owren's Veronal Buffer and test in triplicate, just as a patient plasma. Compare manufacturer's assay value of control plasma to that predicted by test technique. The two values should agree within approximately ± 15%. If poor agreement is found, prepare a new assay curve.

b) Dilute patient plasma 1:10 with Owren's Veronal Buffer (0.1 ml of plasma to 0.9 ml of Buffer).

c) Perform steps b) through l) of Test Procedure - Standard Curve.

NOTE

If clot is not detected, as evidenced by a continually running timer, fibrinogen value is probably very low. Perform step b) 2) of Interpretation of Test Results.

INTERPRETATION OF TEST RESULTS

a) Determine fibrinogen assay value by locating average time
   of patient or control on vertical axis of assay curve
   (Figure 8-5), and read fibrinogen value off horizontal axis.

CAUTION

Mistakes can be easily made in reading
log-log graphs. Therefore, care should
be exercised when reading the fibrinogen
value on the curve.

b) Use center of calibration curve for most accurate results.
   When fibrinogen values are less than 130 mg/dl or greater
   than 700 mg/dl, dilutions should be prepared that will
   provide results in center of curve.

   1) When fibrinogen value is above 700 mg/dl, dilute
      patient plasma 1:20 (0.05 ml plasma to 0.95 ml
      Owren's Veronal Buffer) and rerun test. Multiply
      fibrinogen value predicted by assay curve by 2 to
      obtain true fibrinogen value.

   2) When fibrinogen value is below 130 mg/dl, dilute
      patient plasma 1:5 (0.1 ml plasma to 0.4 ml Owren's
      Veronal Buffer) and rerun test. Divide fibrinogen
      value by 2 to obtain true fibrinogen value.

Fibrinogen Assay Graph Paper

TIME (sec) vs **FIBRINOGEN** $\left(\text{mg}/_{dl}\right)$
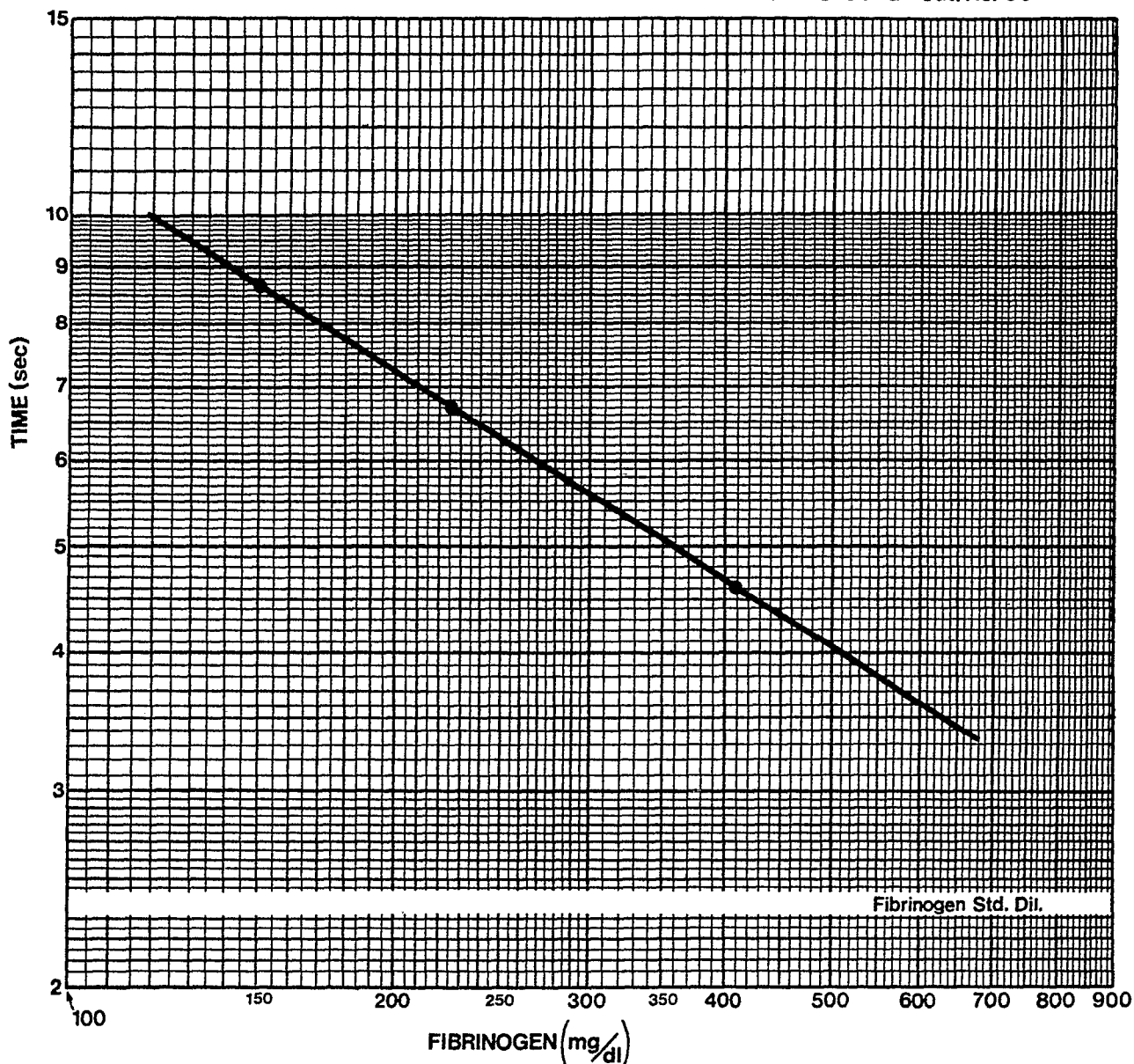
Fibrinogen Std. Dil.

NOTE; Use this graph only with: Fibrinogen Secondary Standard LOT No. _____ mg/dl

Thrombin Time Curve for Fibrinogen Assay. Typical Curve for ELECTRA _____ S/N _____

8—5

ANTITHROMBIN III ASSAY USING THE ORTHO ANTITHROMBIN III ASSAY KIT

PRINCIPLE

Antithrombin III (AT III) is a naturally occurring component of the coagulation mechanism which acts to neutralize activated clotting factors such as thrombin, IXa, Xa, XIa, and XIIa. A deficiency of AT III can be hereditary, such as in Familial Thrombophilia, or acquired, such as in liver disease or disseminated intravascular coagulation (DIC).

Antithrombin III levels in plasma can be measured by determining the amount of thrombin that is inactivated by the defibrinated plasma.

This procedure is a two-stage clotting assay. In stage one, a known concentration of thrombin is partially neutralized by adding it to a heat defibrinated plasma sample. Stage two involves removing a volume of this thrombin/plasma mixture and adding it to Fibrinogen Reference Plasma. The resulting clotting time is compared to a standard curve to obtain the antithrombin III concentration as a percent of normal activity.

This procedure is specifically written to be used with the Ortho Antithrombin III Assay Kit. While other kits are available, at the time of this printing, this is the most widely used AT III clotting assay kit. Procedures for other kits are similar.

MATERIALS REQUIRED

    Electra 750
    0.1 ml Instrument Pipette
    0.2 ml Pipette - MLA #1056
    1.0 ml Pipette
    2.0 ml Pipette
    5.0 ml Pipette
    Pipette Tips - MLA #9620
    Test Cuvettes - MLA #9005
    10 X 75 mm Plastic Tubes
    12 ml or 15 ml Conical Plastic Centrifuge Tubes
    56°C ± 0.5°C Waterbath
    Stopwatch
    Test Tube Rack
    Semi-Log Graph Paper (provided in Ortho Kit)

REAGENTS REQUIRED

    0.85% Saline Solution
    Ortho Antithrombin III Assay Kit containing:
        Ortho AT III Standard Plasma
        Ortho AT III Thrombin
        Ortho AT III Fibrinogen Reference Plasma
        Ortho AT III Plasma Diluent
        Ortho AT III Fibrinogen Buffer

TEST PROCEDURE

PREPARATION OF THE STANDARD CURVE MODULE

a) Remove the Standard Curve Module, Fibrinogen Buffer and one
vial of Plasma Diluent from the Kit.

NOTE

All reagents should be allowed to warm to
room temperature (20° to 25°C) for at
least 30 minutes before reconstitution.

b) Reconstitute the reagents according to the manufacturer's
instructions. Pool the four vials of Standard Plasma in a
labeled 10 X 75mm plastic tube.

c) In labeled 10 X 75mm tubes prepare dilutions of the Standard
Plasma according to the following table:

TABLE 8-5

AT III STANDARD CURVE PLASMA DILUTIONS

TUBE NO.	1	2	3	4	5	6
STANDARD PLASMA	0ml	0.1ml	0.3ml	0.3ml	0.5ml	0.5ml
PLASMA DILUENT	2.0ml	1.9ml	1.7ml	1.7ml	1.5ml	1.5ml
% ACTIVITY	0%	25%	75%	75%	125%	125%

NOTE

The 75% and 125 % dilutions are
prepared in duplicate.

TEST PROCEDURE - STANDARD CURVE

a) Turn on Electra 750. Warm up for 5 minutes or until "AT TEMP"
lights, whichever is later. Perform abbreviated O.C.T.
See section 5.

b) Set Mode Switch to THROMBIN and Lamp Level to B.

c) Prepare Standard Curve Module according to instructions.

d) Test each dilution in duplicate.

e) Pipette 0.4 ml of the Standard Plasma dilution into an MLA
cuvette. Place the cuvette into incubation station #1 and
start the stopwatch.

f) Pipette 0.2 ml of Fibrinogen Reference Plasma into a second
MLA cuvette; and at 2 minutes place the cuvette into
incubation station #2.

g) At 3 minutes add exactly 0.1 ml of room temperature Thrombin to the plasma dilution in station #1 (step e) and reset the stopwatch.

h) At 2½ minutes remove the Fibrinogen Reference Plasma from incubation station #2 (step f) and place it in the test station.

i) Using the 0.1 ml instrument pipette (blue top) remove 0.1 ml of the mixture from incubation station #1 (step g), and align the pipette over the test station. At exactly 3 minutes, firmly push the plunger and hold down for one second to start test.

<u>CAUTION</u>

> Step i) should be carried out in one continuous motion, with the pipette arriving at the aligned position ready to deliver at exactly 3 minutes. This will minimize any change in temperature of the thrombin/plasma mixture.

j) When the timer stops, record the clotting time on the Ortho Data and Calculation Sheet (DCS).

k) To obtain the duplicate clotting time, repeat steps e) through j) using the same plasma dilution.

l) Repeat steps e) through k) for each plasma dilution.

m) Use a clean pipette tip for each step to prevent carry-over contamination.

n) Duplicate agreement should conform to the limits in Table 8-6. If duplicate clotting times do not agree within the specified limits, the test must be repeated for that dilution, in duplicate. Use only the results of the repeat test.

<u>TABLE 8-6</u>

<u>AT III ASSAY ACCEPTABLE DUPLICATE AGREEMENT</u>

CLOTTING TIME RANGE (SECONDS)	DUPLICATE AGREEMENT (SECONDS)
6-12	± 1.0
13-25	± 2.0
26-44	± 3.0
>44	± 4.0

PLOTTING STANDARD CURVE

a) Average the duplicate clotting times and record the aver
on the Ortho DCS. If an average clotting time of less tha
six seconds is obtained, investigate all test system
variables before repeat testing. Record only the results
of a repeat test with an average clotting time of six
seconds or greater.

b) The standard curve is plotted on semi-log graph paper with
the clotting time in seconds, on the vertical (log) axis
and Uncorrected % AT III on the horizontal (linear) axis.

c) The connected points should approximate a straight line.

PATIENT SPECIMEN PREPARATION

a) Within 30 minutes of collection, prepare platelet-poor
plasma from the patient's citrated whole blood specimen.

b) Defibrinate the plasma as follows:

1. Place 1.0 ml of plasma to be tested, equilibrated
to room temperature, into a 12 or 15 ml conical
centrifuge tube and cover tightly.

2. Place the tube in a 56°C ± 0.5°C waterbath for exactl
5.0 minutes.

CAUTION

The temperature of the hot waterbath and
the timing of the heating period are
critical. Heating for a different time
period or temperature than indicated will
yield erroneous test results.

3. Immediately transfer the tube to an ice water bath for
5.0 minutes.

4. Centrifuge the heat treated plasma at 1500-2000 x g
for 15 minutes. Carefully remove the supernatant
defibrinated plasma to a plastic tube. Recentrifuge
at a higher speed if necessary to obtain complete
separation.

5. Test within 1 hour.

TEST PROCEDURE - PATIENT SPECIMENS

a) Remove one Patient Test Module. Reconstitute reagents according to the manufacturer's instructions. This provides sufficient reagent to do duplicate tests on five patients and the defibrinated control provided with each Patient Test Module.

b) Dilute 0.4 ml of control or defibrinated patient plasma with 1.6 ml of Plasma Diluent.

c) Perform steps d) through n) of the Test Procedure - Standard Curve for all control or defibrinated patient plasmas.

INTERPRETATION OF RESULTS

a) Duplicate clotting times for patients and controls should agree within the limits defined in Table 8-6. If they do not, the results of repeat tests should be used.

b) Average the clotting times for each plasma dilution and read the Uncorrected % ATT III from the Standard Curve.

c) Patient results are corrected for module to module variation by using the procedure outlined in the Ortho Kit package insert.

d) If a sample yields a result greater than 125%, repeat the test in duplicate using a sample of 0.2 ml defibrinated plasma and 1.8 ml Plasma Diluent. The % AT III of this dilution should be doubled to determine the actual % AT III for the sample.

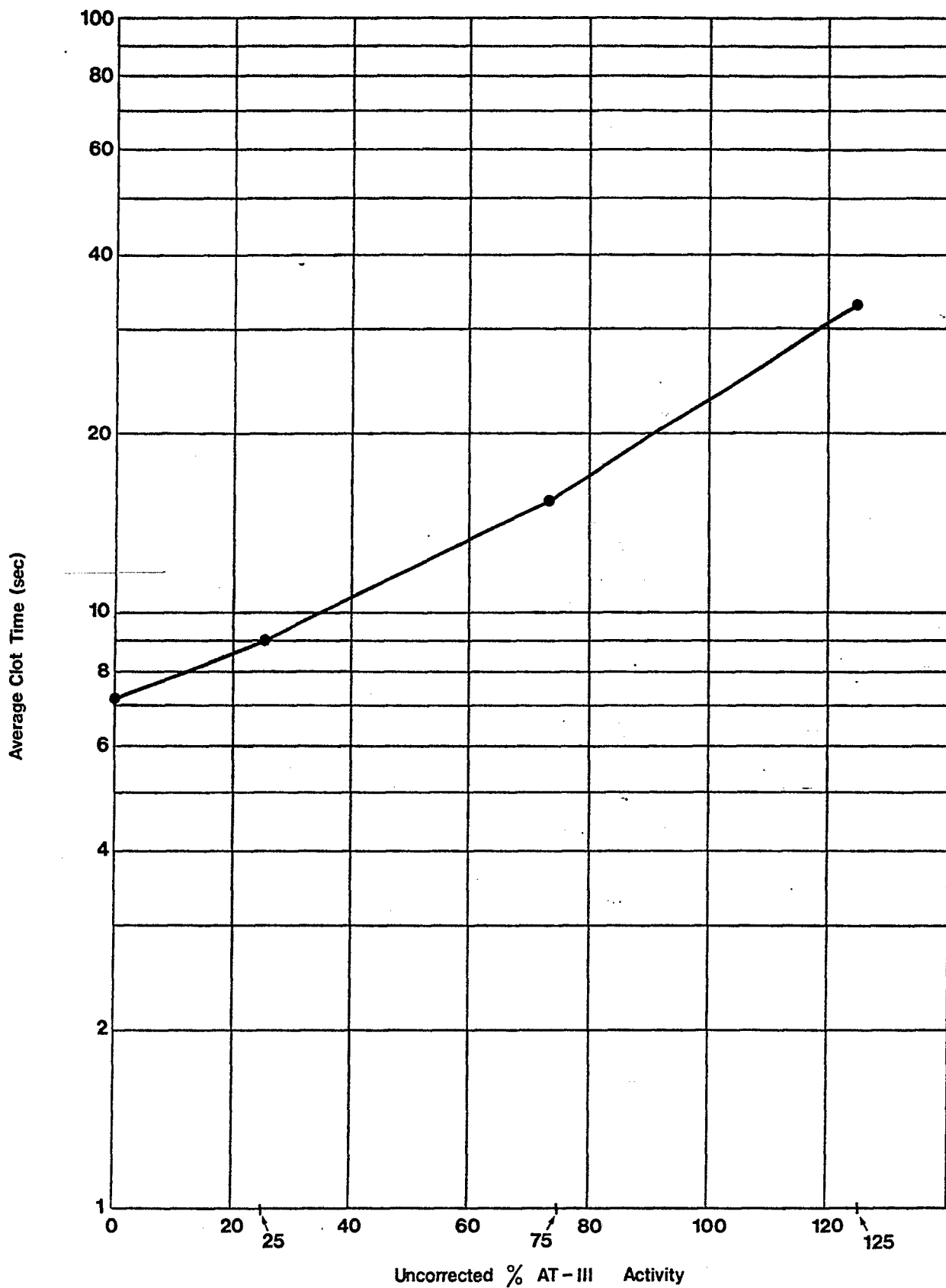e) Corrected % AT III values should be reported with a laboratory established normal range.

Figure 8-6

Typical Antithrombin III Assay Standard Curve

INSTRUMENT CORRELATION

INTRODUCTION

A number of different techniques are in use today for measuring the clotting time of plasma. The most commonly used are photo-optical and electro-mechanical. The clotting times by these different methods (and even by different types of instruments within a category) will not usually be identical. Since there are no true standards in coagulation at this time, one cannot assign a "correct" time to any coagulation test. Each laboratory must strive to achieve uniform results within the laboratory and results comparable to other laboratories using the same system. While the different types of coagulation instruments may be shown to give different clotting times under controlled testing situations, the other variables in the system (reagents, lab techniques, etc.) usually contribute to an even greater variance.

COMPARISON OF TWO TECHNIQUES

In general, the following elements are common to all coagulation procedures:

1. Drawing of the patient's blood sample into an anticoagulant.

2. Storing the patient sample, transporting to test site, centrifuging and storing the patient's plasma prior to the clot time testing.

3. Inserting a standard quantity of patient's plasma into a vehicle for conducting the time test.

4. Initiating the clotting action (and start of timing) by the introduction of a reagent.

5. Detecting the incidence of clotting and coincidental termination of timing.

It is important, in making comparisons of alternate clotting procedures, that the above factors be identical for the various techniques. The equivalence of alternate procedures must be assured, since blood clot timing characteristics of any blood sample can be altered by the following:

1. The vehicle in which it is collected, stored, and tested.

2. The temperature during storage, transporting, incubating and clotting observation.

3. The nature of the reagent - the manufacturer, the uniformity between batches; the age of the reagent and length of time since reconstitution; the accuracy of reconstitution.

4. The volumetric ratios of plasma and reagent in the test vehicle.

5. The treatment of the test sample in exposing it to motion during testing or to contact with the clot sensing device.

Simultaneous testing with alternate methods for reasons of comparison of clot times, should be handled as follows, and as many variables as possible should be eliminated:

1. Patient's samples should be taken from the same collection and centrifugation container and be subjected to identical handling in preparation for insertion into the clot timing device. For APTTs especially, incubation time, temperature and handling must be identical.

2. Pipetting of the standard test sample should be done by the same operator with the same pipette. Pipette tips must be changed between patient samples.

3. The reagent should be taken from the same prewarmed supply and the dispensing volumetric accuracies must be assured for both procedures.

In order to compare two techniques, both patient plasmas and control plasmas should be run over several days according to the procedure outlined above. The utmost care must be taken to perform each technique precisely. At least 25 to 30 samples should be tested in duplicate with each technique. The clot times should then be plotted in a fashion similar to that shown in Figure 9-1. Such a graph can then be used to relate clot times from one system to the other.

COMPARISON OF ELECTRA 750 AND ELECTRA 600

Figures 9-1 and 9-2 show the correlation between clot times for the Electra 600 and the Electra 750 for PT and APTT tests, respectively. These graphs show typical performance for the instruments being used according to procedures contained in the Operator's Manual for each instrument. These graphs may change somewhat depending upon reagents used or variations in technique.
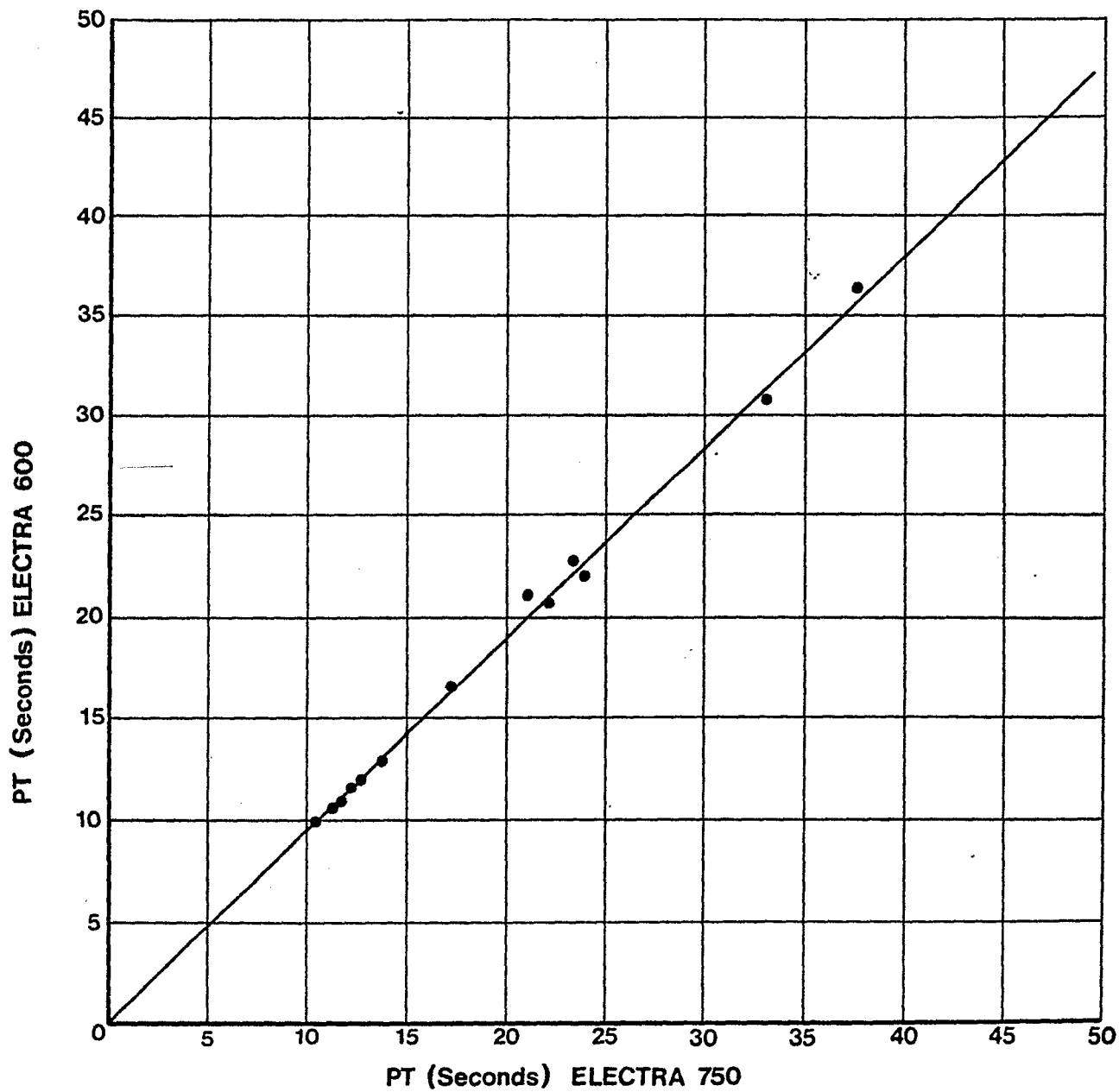
9-1   Correlation Between 750 & 600 for PT Tests
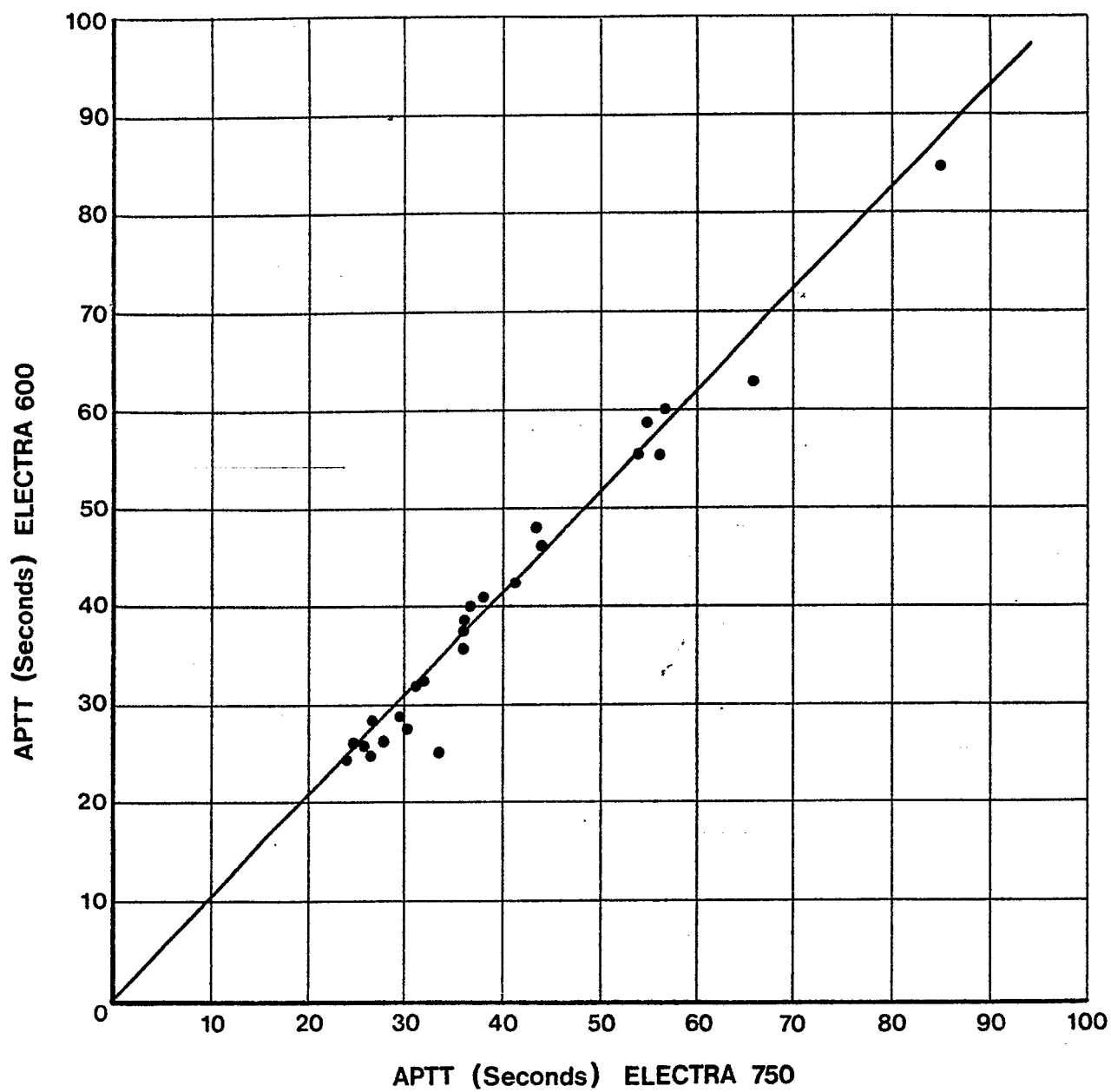
9-2   Correlation Between 750 & 600 for APTT Tests

SECTION 10

MAINTENANCE

PREVENTIVE MAINTENANCE

There are no specific preventive maintenance procedures that must be followed. However, normal cautions should apply.

Take care not to spill reagent or plasma in the work station.

Keep work station clean if spills occur.

Wipe face plate and case to remove fingerprints and foreign matter.

Check for accumulation of debris in test station and heating wells.

CHANGING PHOTOLAMP

a) Disconnect primary power (pull plug).

b) Remove eight screws holding cover.

c) Remove Printed Circuit (PC) board clamp and PC board indicated in Figure 10-1.

d) Disconnect lamp leads and unscrew knurled screw holding photolamp.

e) Remove photolamp.

f) Insert new photolamp so that it is properly seated with flange against mounting block, tighten thumb screw firmly and connect lamp leads.

g) Replace PC board and clamp removed in step c). Then replace case and screws. Reconnect primary power.

1. REMOVE PC BOARD CLAMP

2. REMOVE THIS BOARD

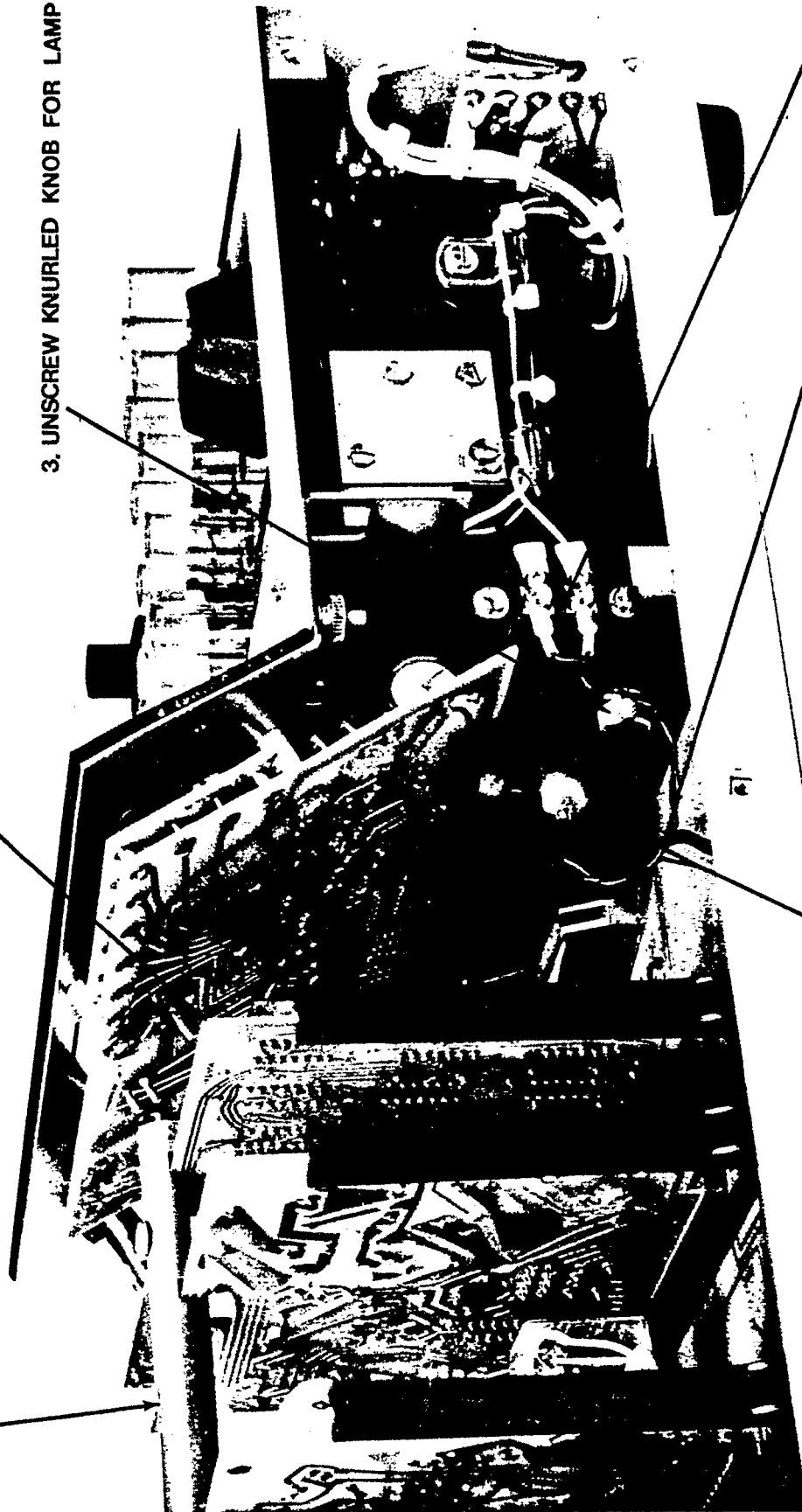3. UNSCREW KNURLED KNOB FOR LAMP REMOVAL

4. REMOVE THESE WIRES AT THIS POINT

5. REMOVE PHOTOLAMP ASSEMBLY

Figure 10-1 Photolamp Replacement